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Aquatic Mammals 1989, **15.3,** 85–86

Editorial, News and Views

It has always been the intention of this Editor that authors, whose papers have been accepted for publication, should not have to wait too long before these see the light of day. Latterly, due I am glad to say to an influx of interesting and acceptable papers, the gap between acceptance and publication threatened to increase to one year and over. Obviously, one answer would be to print an extra issue, but, at the present subscription level, kept on purpose low to cover only printing and distribution costs, and with no financial reserves, this would just not be possible without running the finances into the red. As these are my sole responsibility—acting without the backing of any organisation—I would be responsible for any costs incurred by an overdraft. I am therefore delighted to announce that, at no cost to yourselves as Subscribers, there will be four issues comprising Volume 15. This has only been made possible by a generous grant from the Harderwijk Marine Mammal Park, who incidentally celebrate their 25th Anniversary in 1990. Not surprisingly the issue contains papers based on research work carried out either at the Park or by their staff. If any other Organisation wishes to instigate a similar proposition, I am sure they can be accommodated.

The Paper (on p. 95) by Duinker and his co-workers, gives a very detailed account of the relative importance of some pollutants in cetacean species in both the North Sea and the Atlantic Ocean. It was noted that the apparent rates of biotransformation of these pollutants were higher in seals than in the cetaceans examined. This finding alone seems important and relevant in view of the suggestion, often voiced, that the Morbillivirus infection, recently seen to have such a disastrous impact on the survival of seals in the North Sea, may have taken greater hold due to an increase in the levels of pollutants in the oceans. In such a detailed paper, the technical side is very important to give credence to the findings, although to many of us I suspect it is too specialised for full appreciation and comprehension. However the findings are definitely of interest to all of us and certainly worth reading with care and digesting.

In Volume 15, No. 2, I referred to the report in 'The Pilot'—the newsletter of the Marine Mammal Action Plan—of mysterious deaths of Bottlenose dolphins, Humpback and Minke Whales off the East coast of North America. Their latest issue, number 3, identifies the cause as a brevetoxin. This is produced by an algal species which forms large 'blooms' under favourable conditions of weather. Freak currents then carried these 'blooms' from the Gulf of Mexico to the coast of Florida. Fish then ate the algae but the toxin remained in their gut. Consequently gutted fish eaten by man did not cause trouble, but the whole fish swallowed by Cetaceans caused absorption of the toxin, affecting the liver and the animals' ability to fight against secondary infection. Yet another example, I suppose, of the wild not being the benign benevolent environment some people would have us believe.

In the same number, the Editorial rejoices in the Plan (MMAP) having 'found its feet' but at the same time criticising the lack of coordination around the world between differing bodies trying to help conservation on their own and in their own way. It cites as an example the many well-meaning activities designed to aid the Mediterranean Monk Seal—from large bureaucracies (like the Berne Convention) to private initiatives (like the Fund for the Mediterranean Monk Seal in Palma de Mallorca). Unfortunately until the MMAP takes overall responsibility, the species is dwindling in numbers while helpers are 'running in circles' round the animals.

On the same theme, it records that the Nature Reserve for the Isles Desertas, S.E. of Madeira, intended to protect the 8–12 Seals there, has still not been set up. It appears that political pressure from fishermen has prevailed over that wielded by conservationists. The few animals therefore remain vulnerable. In 1985, Vol. 11, No. 2, and again in 1987, Vol. 13, No. 3, I drew readers' attention to the misuse of dolphins to provide meat for the baiting of crab traps—instead of the legal and recommended use of sheep meat. Now we read that the Commerson's Dolphin is so scarce that crab fishermen are looking to use Peale's Dolphin, the Dusky Dolphin, Southern Sealion, Southern Fur Seal or several species of Penguin. It has always seemed to me that there is no point in having laws on the Statute Books, or even signed International Agreements, without the full agreement of all parties or adequate policing of the arrangements. Here is yet another example where the intention is right but the ability to enforce is lacking. Once the above species have been decimated, where to next?

The Journal of Zoo and Wildlife Medicine, Vol. 2, No. 1, includes a very interesting paper on the evaluation of dietary husbandry of marine mammals. At the pools investigated, it was not too surprising to learn of substandard levels of Vitamin E being fed, but what was perhaps to many of us more so, an excessive supplementation of both Vitamins A and D. It has long been known that both can be toxic in high doses so the

finding was very enlightening. It also mentioned the excessive use of common salt (Sodium chloride) in the fresh water environments of Pinnipeds. It offered advice on the remedies to put the situation right—both from the view of the health of the animals and to save labour and costs.

The Seal Mammal Research Unit at Cambridge is using a French based satellite in its telemetering studies to monitor the physiology and behaviour of Pilot and Sperm Whales and of Grey Seals. The telemetry units they find to be long lasting and this is illustrated by a photograph of a grey seal in the wild, with such a unit still attached some 10 months later.

On the same front, radio tracking of Florida Manatees has been achieved for several years now and recently four Australian Dugong have been tracked for up to 11 weeks. These latter animals, it is interesting to note, only moved some 4–11 kms. from the core area during this period. Such methods are to be encouraged if their use prevents well meaning conservationists disturbing the environment of such timid and highly specialized feeding animals just to count them!

The International Association for Aquatic Animal Medicine, who met in San Antonio earlier this year, announce that the 1990 meeting will be held in Vancouver, British Columbia, and that plans are afoot to hold the 1991 conference in S. Augustine, Florida. IAAAM News includes a very useful list of Current Literature and Book Reviews and also a list of positions available and funding opportunities.

Reports in England suggest that the Seal 'Distemper' may be about to strike again. The first dead pups have recently been washed ashore. On the same subject, The Veterinary Record, Vol. 124, No. 19, records the isolation of Mycoplasms from seals affected with the Morbillivirus from both the North and Baltic Seas. Is there ever such a thing as a single agent condition?—perhaps excluding Anthrax.

Don't forget anyone wishing to buy books, especially on the Marine Mammal field would do well to contact Chiron Publications, P.O. Box 25, Keighley, West Yorkshire, U.K. in the first instance to obtain what will probably be a very favourable quote.

Aquatic Mammals 1989, **15.3**, 87–88

Letters to the Editor

Sir,

The biological sonar system of odontocetes allows them to gather relevant information about their surroundings: obstacles, food and accompanying animals. Their sonar system can be either a passive one or can be actively used. In the latter case the animal is echolocating in its environment by means of a specifically structured acoustic signal. In this way it uses both its sound-producing capabilities and its auditory system to reveal acoustic features that are of prime interest.

Limitations on the performance of this active process of echolocation are determined not only by the perceptual faculty of the animal, but also by the specific emitted signal. The auditory sensitivity of odontocetes has been a subject of thorough investigation since 1966. It does appear that the established excellent auditory capabilities to perceive signals in the order of up to some 150 kHz give sound reasons to stipulate that the used echolocation frequencies are in the same frequency range (Dubrovskii *et al.*, 1971; Popper, 1980).

However, our knowledge and understanding of dolphin sonar is far less complete than our knowledge of dolphin hearing (Ridgway, 1982). Maybe the reason could be the lack of—expensive—equipment in the laboratory and in the field or maybe it is a different approach on the part of researchers to the understanding of sonar.

In any case, looking for an answer in literature, we note an extensive interest in theories about the mechanism of sound production and only minor attention to the sonar signal problems, although there should be a close relation between the two. We need to fill up the gaps in sonar descriptions, especially in the case of wild dolphins. Therefore, it is disappointing to have to state that several opportunities to obtain high quality recordings were missed. To cite one of them: Morris & Lockyer (1988), who studied a juvenile wild bottlenose dolphin SIMO, made 'a range of underwater recordings of the animal's underwater acoustic emissions'. These recordings were made with a NAGRA IVSJ, covering a recording bandwidth up to 35 kHz, whereas Morris on page 387 in Bryden & Harrison's *Research on Dolphins* (1986) indicates: 'dolphins emit up to 200 kHz'.

In fact, the 35 kHz bandwidth recording apparatus then causes the dolphin's high frequency sonar sound to be subjected to some kind of a low-pass filter with a grossly nonlinear amplitude response. Probably, the authors did not grasp the simple advice on underwater sound recording (as was already brought forward in 1973) in the articles of the JASA by Diercks, Trochta & Evans: 'Adequate bandwidth is the prime requisite for validity in (delphinid sonar) measurements' and, more recently, the self-evident remarks of Watkins & Wartzok (1985): 'good bioacoustic records need among other things the use of equipment suitable in bandwidth, dynamic range, sensitivity and cable impedance for the particular recording intended'.

Maybe we should not only complete the list as compiled by Watkins & Wartzok, but add to the

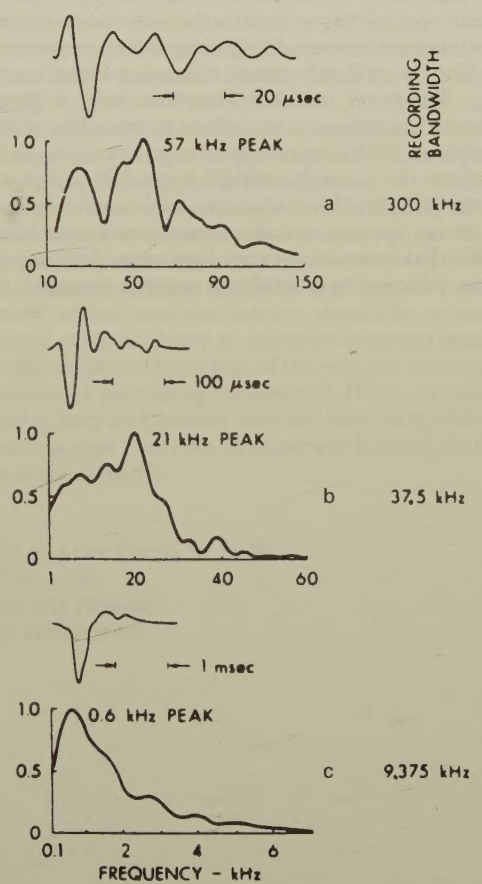

Figure 1. Demonstration of effects of inadequate bandwidth recording. Top: simulated dolphin-like click. (a) Nominal bandwidth; (b,c) Reduced bandwidth recording. Taken from *JASA* (1973), **54(1)**, p. 202.

validity of the sound recordings with a note on the bandwidth.

The effects of recording with inadequate bandwidth are clearly illustrated in Figure 1, where a simulated dolphin-like click is submitted to recording at reduced bandwidth. Figure 1b would then easily lead to an explanation for the description of a click as: 'complex, structured, broad-band pulse containing most energy between 6–24 kHz' (Morris & Lockyer, 1988). Or perhaps, do we face here different acoustic behaviour from SIMO in British waters as opposed to that of Jeanne Louise along the coast of Brittany? (Kamminga, in press).

It is a pity to have to state that the cited example is not the only one, although it is the most recent one in quite a list. A hundred to one chance was missed in getting a perfect full bandwidth recording of *Lipotes vexillifer* (the Chinese river dolphin) when Jing Xianying *et al.* (1981) (p. 408) recorded underwater acoustic signals with a frequency bandwidth of up to 30 kHz. Let us hope that in the near future there will be an opportunity to register the entire acoustic spectral range of this rare dolphin species in a proper way.

The marvellously filmed Cousteau expedition to the Amazonas in 1982 illustrates how a special chance was missed in the full range recording of wild dolphins, held temporarily in captivity. Even in the eighties the audio bandwidth limited NAGRA still seems to be the ultimate in ultra-sonic sonar recording

In my opinion, the above-mentioned points illustrate that more cooperative interaction between different disciplines in delphinid research is needed; this type of research must be indeed multidisciplinary! Let us not deny that an adequate description of acoustic behaviour does not end with a qualitative description but also comprises biochemistry, psychophysics, biomathematics and much more.

C. KAMMINGA

References

Diercks, K. J., Trochta, R. T. & Evans, W. E. (1973). Delphinid sonar: measurement and analysis. *J. Acoust. Soc. Am.* **54(1),** 200–205.

Dubroviskii, N. A., Krasnow, P. S. & Titov, A. A. (1971). On the emission of echolocation signals by the Azov sea harbour porpoise. *Soviet Physics—Acoustics,* **16(4),** 444–448.

Jing Xianying, Xiao Youfo & Jing Rongcai (1981). Acoustic signals of the Chinese river dolphin (*Lipotes vexillifer*). *Scientia Sinica* **24(3),** 407–415.

Morris, R. J. (1985). The acoustic faculty of dolphins. In: M. M. Bryden & R. J. Harrison (eds), *Research on Dolphins.*

Morris, R. J. & Lockyer, C. (1988). A juvenile wild bottlenose dolphin. *Aq. Mamm.* **14(2),** 49–63.

Popper, A. N. (1980). Behavioural measures of odontocete hearing. In: R. G. Busnel & J. F. Fish (eds), *Animal Sonar Systems.* New York, Plenum Press, pp. 469–483.

Ridgway, S. H. (1983). Dolphin hearing and sound production in health and illness. In: R. R. Fay and G. Gourevitch (eds) *Hearing and other senses: Presentations in honour of E. G. Wever.* The Amphora Press, pp. 247–297.

Watkins, W. A. & Wartzok, D. (1985). Sensory biophysics of marine mammals. *Marine Mammal Science* **1(3),** 219–260.

Book Review

HARPS AND HOODS—Ice-breeding seals of the Northwest Atlantic. David M. Lavigne and Kit M. Kovacs, University of Waterloo Press, Waterloo, Ontario, Canada. 174+xviii pp. £25.

At first glance, this seems to be another book about cute, furry little seals, with a lot of pictures and no information. This first impression is totally wrong. It does have a lot of colour photographs, but the information given in the book is substantial.

In the introduction there is an outline of the taxonomy of pinnipeds and short descriptions of the harp seal, *Phoca groenlandica*, and the hooded seal, *Cystophora cristata*. The second chapter briefly deals with distribution and migration, basically to put the seals' life cycles in their geographical context, and then the authors turn to a thorough description of the life cycle. First a detailed account is given of the harp seal, in which most attention is paid to the subadult stages. All of these stages, from yellowcoat through whitecoat, beater and bedlamer to spotted harp, are explained and the origins of their names are dealt with as well. There is only a short description of the adult stage. After this a similar but shorter account is given of the hooded seal.

The next chapter deals with their adaptations to life in the sea. A lot of attention is paid to the problems associated with diving. In fairly simple language the physiology of diving and research in this field are explained. When discussing the sense organs the authors concentrate on vision and, using several diagrams, explain the problems of amphibious vision. Other aspects of physiology are dealt with briefly.

In the fifth chapter an overview is given of the history of the Northwest Atlantic seal hunt. The overview starts with the 16th century walrus hunt and goes from there to the grey seal hunt and the discovery of the ice-breeding seal populations in the 17th century. From there a detailed account is given of the several changes in hunting techniques and the impact those changes had on the populations. A large part of this chapter deals with the period from 1964 to now. The year 1964 signified a change in the public awareness of the seal hunt. In that year a television documentary on the seal hunt was broadcast which essentially triggered the anti-sealing movement, led by Brian Davies. Nineteen-hundred and sixty-four also marked the start of a strict regulation of the seal hunt, and subsequent Greenpeace involvement (since 1976) in the sealing controversy led to an even higher public awareness. Elaborating on the arguments in the last part of the fifth chapter, the authors state in conclusion that the future of the commercial seal hunt hinges not on scientific arguments about population control, but on the availability of markets for seal products. The book ends with a reference of selected reading and a glossary. Throughout the book, words that are explained in the glossary are printed in bold type.

This book is a very readable and brilliantly illustrated account of the natural history of two seal species, which are subject to a lot of controversy. The last chapters dealing with the seal hunt are very clearly written and the authors should be complimented on their ability to maintain a neutral stance on this subject. This is one of the very few unbiased accounts of the sealing controversy. If you are interested in harp and hooded seals and want to be able to make up your own own mind on seal hunting, this is the book to read.

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Some further observations on blood from a captive California Gray Whale, *Eschrichtius robustus*

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The whales (order Cetacea) comprise a group of marine mammals that are divided into three suborders: the *mysticeti* (baleen whales), the *odontoceti* (toothed whales), and the *archaeoceti* (an extinct group). The suborder *mysticeti* contains the largest animals that have lived (McVay, 1966; Rudd, 1956). These large mammals have evolved numerous physiological adaptations to their harsh and cold environment. Two major adaptations were the loss of body hair and the accumulation of large amounts of subcutaneous fat filled blubber used as insulation to aid in maintaining body temperature and as a source of energy for long migrations. This accumulation of blubber has long been exploited by whalers for its oil. The once relentless hunting for whale oil has in the past driven some whales to near commercial extinction. This certainly is true for two baleen whales, the Blue whale (*Balaenoptera musculus*) and the Bowhead whale (*Balaena mysticetus*) (McVay, 1973). A third species, the California Gray whale (*Eschrichtius robustus*) was hunted to near extinction but has been protected by international treaty for the last 40 years and has recently made a dramatic recovery to its original numbers (Gilmore, 1955; Walker, 1971).

This report concerns a Gray whale calf (Gigi) that was kept in a marine zoological park for one year and then released (Fig. 1). An earlier attempt to keep a Gray whale calf in 1965 failed as the animal died after two months in captivity (Wahrenbrock, 1974). The only other baleen whale documented and maintained for study or display was a Minke whale in Japan (Defran & Pryor, 1980).

Many important physiological studies were made on Gigi resulting in a special publication of the *Marine Fisheries Review* (Evans, 1974). Since her release, nothing more has been published about her physiological status during that year of study. Through the cooperation of Sea World, San Diego, some additional results of the year-long blood monitoring programme have been made available. The trends observed during the course of the year are the basis of this report. Data for the middle 6 months of her stay are not at Sea World and were thus not available to us.

Blood studies on baleen whales, with a few exceptions (Zettner, 1974; Medway, 1974; Gilmartin *et al.*, 1974; Spencer, 1973; DeMonte & Pilleri, 1971, 1979*a*, 1979*b*, 1982), are scarce. We hope this report will be useful to the further understanding of these animals and their habitat (Mitchell, 1973).

Materials and Methods

A California Gray whale calf (Gigi, estimated age 10 weeks) was captured on 13 March 1971 in Scammon's Lagoon and arrived at Sea World, San Diego four days later. There she was maintained for one year and then released on 13 March 1972. During this period, her blood was sampled and studied many times. Blood was collected in KEDTA tubes for haemographic studies and in clean glass tubes for serum chemistry analysis. Other studies were also conducted as reported previously (Evans, 1974).

Automated and discrete systems were used for the analyses either at the Sea World laboratory or were sent to local commercial laboratories.

Results

The results are shown in Tables 1, 2, 3, 4 and 5.

Table 1 has the available results of haemographic studies. Note the differences between the initial sample and the final sample.

Table 2 shows the results of the routine blood metabolite analyses. These are rather sketchy; however, they do illustrate trends that took place.

The sodium levels are consistently in the low normal range for marine mammals and could be indicative of the mostly squid diet which is high in water content and relatively low in sodium (Sidwell *et al.*, 1974).

Table 3 shows the results of the lipid analyses over a period of 20 days (3 samples) during the mid-portion of her year of study.

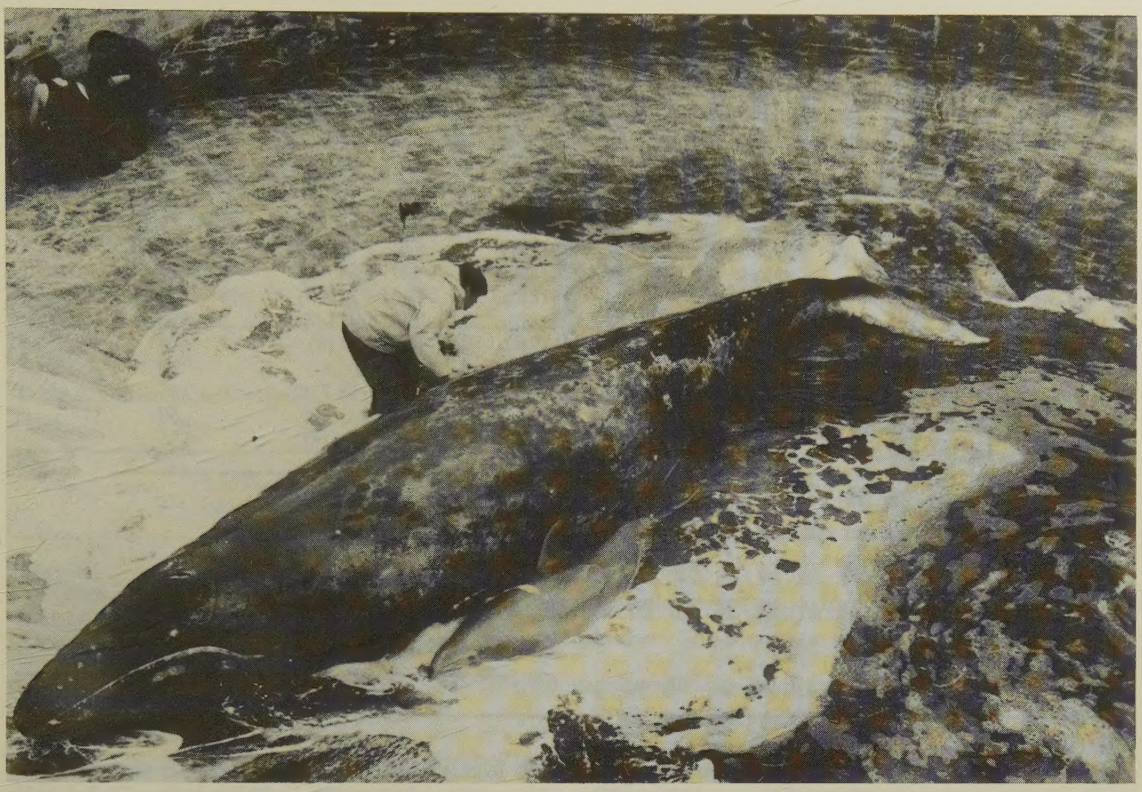

Figure 1. The young whale (Gigi II) is stranded in the pool for sample collection and examination. Note the size difference between Gigi and her pool mate, a mature bottlenose dolphin.

Table 4 gives the results of a number of protein separations by chemical means.

Table 5 shows the results of electrophoretic separation of the serum proteins. The dates of collection are indicated.

Discussion

On examination of the data in Table 1, the marked improvement of the haemographic picture from the first day of sampling (18 March 1971) to the last (13 March 1972) is readily apparent. Improvement was noticeable in all parameters. The animal was slightly anaemic initially which, over the course of the year, was resolved. Since these results are from but one whale, whether this was a true anaemia or a physiologic anaemia is not known. Physiologic anaemia is seen during the changeover from placental to alveolar respiration. This occurs in most domestic species at some early age depending upon the species (Schalm *et al.*, 1975) but is not usually lengthy. The animal underwent 3–4 days of transport before arriving at Sea World. The anaemia and the slight leukocytosis on the day of arrival could have been a response to this transport, which is likely as shown by the left shift in neutrophils, a moderate lymphopaenia and an eosinopaenia. Some of these changes could also be due to a mild infection—the result of exposure to the terrestrial microorganisms she encountered for the first time. While no eosinophils were enumerated in any of the haemograms nor were any identified on the blood smears, this does not imply that none were observed on scanning the smear. In our experience, the MCV was also somewhat decreased.

Over the first 3 months, improvement was gradual but continuous. There were several unusual husbandry episodes during this period as we learned new technology almost daily.

The data from the final 3 months indicate a continual improvement toward what, in our experience, one would expect in an adult whale. Why the MCV was only 117 femtolitres* during this time is not known. The last sample at the time of release was probably as clinically normal as one could expect, indicating that a healthy, normal whale was released.

*Editors note: 1 femtolitre ≡ 1 cubic micron; 1 microlitre ≡ 10^9 femtolitres.

Table 1. Haemograms on blood from Gigi II during captivity

	1st sample 18 March 1971	First 3 months	Last 3 months[b]	Last sample 13 March 1972
PCV (%)	31	35.8 ± 3.4 (11)[a]	43.8 ± 1.4 (8)	43
Hb (g/dl)	10	12.1 ± 1.6 (11)	15 ± 1.0 (8)	15
RBC × 10^6/µl	2.4	2.8 ± 0.5 (11)	3.79 ± 0.4 (8)	3
WBC/µl	13900	11542 ± 4804 (11)	7036 ± 1267 (8)	5600
S	8957	9464 ± 274 (8)	5277 ± 450 (8)	4704
NS	2641	346 ± 216 (8)	324 ± 211 (8)	0
L	1112	1154 ± 135 (8)	1337 ± 478 (8)	896
M	1390	577 ± 101 (8)	106 ± 91 (8)	0
E	0	0	0	0
MCV (fl)[c]	128	129 ± 18.8 (11)	117 ± 13.7 (8)	143
MCH (pg)[d]	43	45 ± 7.4 (10)	40 ± 5.4 (8)	50
MCHC (%)	32	34 ± 3.6 (11)	34 ± 1.6 (8)	35

[a]Mean ± Standard Deviation (number of samples examined)
[b]No data were made available for the preceding 6 month interval
[c]fl = femtolitres
[d]pg = picograms
Presence or absence of platelets or reticulocytes was not indicated

Table 2. Chemical values on serum from Gigi II during her captivity

	First 3 months initial values Mean ± S.D.	15 Nov 1971	18 Nov 1971	30 Nov 1971	29 Dec 1971	23 Feb 1972
Na (meq/l)	141 ± 6.6 (11)	—	143	—	—	140
K (meq/l)	3.5 ± 0.5 (11)	—	3.7	—	—	3
Cl (meq/l)	102 ± 6.6 (11)	—	118	—	—	102
CO_2 (meq/l)	—	—	19	—	—	25
Ca (mg/dl)	10 ± 1.2 (10)	—	10	—	—	—
Mg (mg/dl)	—	—	1.9	—	—	—
PO_4 (mg/dl)	4.6 ± 0.7 (10)	—	4.9	—	—	—
Glucose (mg/dl)	99 ± 30 (9)	—	92	—	—	102
BUN (mg/dl)	58 ± 9.8 (10)	—	87	—	—	73
Creat. (mg/dl)	1.6 ± 0.5 (9)	—	0.9	—	—	1
T. Bil. (mg/dl)	1.04 ± 0.4 (9)	—	—	—	—	—
Chol. (mg/dl)	852 ± 451 (9)	—	1400	—	1550	—
Fe (µg/dl)	77.5 ± 17 (4)	60	—	120	—	—
TIBC (µg/dl)	525 ± 50 (5)	352	—	350	—	—
% Sat.	13	17	—	34	—	—
ALT[a]	14 ± 6 (9)	—	20	—	—	—
AST[b]	64 ± 35 (10)	—	53	—	—	53
ALP[c]	50 ± 26 (8)	—	74	—	—	87
LDH[d]	387 ± 273 (7)	132	103	112	118	—
CK[e]	96 ± 62 (5)	—	—	—	—	—
Acid Phos.	3.9 ± 1 (2)	—	1.9	—	—	—
Amylase	79 ± 29 (9)	—	—	—	—	—
Lipase	—	—	3.8	—	—	0

[a]ALT = Alanine aminotransferase (formerly SGPT)
[b]AST = Aspartate aminotransferase (formerly SGOT)
[c]ALP = Alkaline phosphatase
[d]LDH = Lactic dehydrogenase
[e]CK = Creatine kinase

Table 3. Results of lipid analyses on serum from Gigi II

	Lipid analysis (mg/dl)			
	26 May 1981	7 June 1971	13 June 1971	X ± S.D.
Chol. (Tot)	1755	1600	1840	1732 ± 122 (3)
Chol. ester	1141	1150	—	1146 ± 6 (2)
Free chol.	614	450	—	532 ± 116 (2)
Phospholipids	1063	1104	—	1084 ± 29 (2)
Total lipids	3238	3338	4400	3659 ± 644 (3)
Triglycerides	135	860	188	394 ± 404 (3)

Table 4. Serum protein values from Gigi II by chemical assay

	First 3 months initial values	Last 3 months
TP (g/dl)	8 (9)[a]	9.5 ± 0.8 (5)
Alb (g/dl)	4.8 (9)	5.6 ± 1.0 (5)
Glob (g/dl)	3.5 (8)	3.9 ± 0.6 (5)
A/G	1.4	1.5 ± 0.4 (5)

[a]( ) Number of samples

In the data received no mention was made of the presence of reticulocytes, platelets or any abnormal red cell morphology. However, Zettner estimated on the basis of a smear, that there were between 300,000 and 350,000 platelets per microlitre which can be considered normal (Medway, 1974). He also stated that the red cells were moderately anisocytotic, a common finding in other cetaceans studied. There was also an occasional polychromatic red cell and rare Howell-Jolly bodies. No nucleated red cells were seen.

It is interesting to note that DeMonte & Pilleri (1982) referring to Zettner's report state that the red cells showed 'marked anisocytosis'—an erroneous conclusion.

When the white cell picture was viewed from the absolute point of view there was little change in lymphocyte numbers over the course of the year. The leukocytic response to external factors by odontocetes is not as marked as it is in domestic species (Medway *et al.*, 1970; Medway & Geraci, 1964; Geraci & Medway, 1973; Ridgway, 1972). How the mysticetes might respond has not been investigated, but one would expect them to react similarly to other cetaceans until proven otherwise.

Initially the total iron and the percentage saturation were below normal or were lower than would be expected in a normal mammal. Most haematologists agree, however, the best test for iron adequacy in the blood is haemoglobin concentration regardless of iron values and iron binding capacity.

With the exception of the rising total cholesterol and the improving iron status of the blood, the non-enzymatic blood plasma constituents (Table 2) were not remarkable. The chemical analysis of blood plasma was rather sketchy; however, of the main constituents measured on 23 February 1972, none showed any evidence of disease. The BUN (Blood Urea Nitrogen) levels are difficult to interpret since the relation between feeding and sampling time is not known. The enzymatic constituents likewise are not remarkable except for LDH (Lactic dehydrogenase) which by itself is of very little value since imperceptible haemolysis can cause erratic levels. (In the clinical experience of one of us with odontocetes [LHC], an elevation in LDH *with the absence* of other enzyme increases, suggests lung inflammation.) The reason for the elevated lipase, 3.8 units, on 18 November 1971 cannot be explained.

The range of total cholesterol during her year of captivity was roughly 400–1840 mg/dl. However, on 19 December 1971 the total cholesterol was 1550 mg/dl. Whether this represents a plateauing or a gradual fall as a result of acclimation and return to good health is not known. The diet initially consisted of high levels of bovine cream, and some thought has been given to the long term effect of the high fat content. Anxiety, stress and long term effect are also known to cause increases in total cholesterol. But there were no other compatible changes and no outward signs of anxiety recorded. Quite the contrary—the whale was continuously playful, alert, and was eating about 2000 lb of food per day, primarily squid, when released. Squid has a very low total lipid content (4.1 gm/lb) and no cholesterol (most fish products are low in cholesterol) (Sidwell, 1974). Most odontocetes ordinarily exhibit higher than the usual normal cholesterol levels as well.

Other lipid constituents of the blood (Table 3) were elevated also. From the concentration of these lipids, the plasma must have been opalescent on gross examination. This is often the case when examining blood from unfasted whales. The total bilirubin determinations were impossible mostly due to the opalescence.

Table 5. Results of electrophoretic separation of serum proteins from Gigi II

	TP	Alb[a]	Glob	A/G	α_1	α_2	β_1	β_2		Sample date
	6.9	4.2	2.7	1.56	0.14	0.97	1.17	—	0.22	18 March 1971
	8.6	5.0	3.6	1.39	0.26	—	1.97	—	1.37	31 March 1971
	9.2	5.25	3.95	1.33	0.18	2.02	1.38	—	0.27	26 April 1971
	9.2	4.8	4.4	1.09	1.6	—	1.56	—	1.2	12 May 1971
	10.2	4.7	5.5	0.85	0.4	1.53	1.84	1.53	0.20	26 May 1971
	9.2	5.25	3.95	1.33	0.18	1.38	1.56	0.64	0.18	4 June 1971
	10.5	6.6	3.9	1.69	2.0	—	—	1.8	0.1	7 June 1971
	10.4	5.1	5.25	0.97	1.54	—	1.65	—	2.06	13 June 1971
	9.0	4.5	4.5	1.0	1.3	1.4	1.4	—	0.4	15 Nov 1971
	9.1	6.0	3.1	1.94	0.72	0.46	1.26	0.54	0.09	30 Nov 1971
X	9.23	5.14	4.1	1.32	0.8	1.29 (6)	1.53 (9)	1.13	0.60	n = 10

[a]All components in g/dl (grams per decilitre).

The results in Table 4 indicates little change in total protein throughout the year. These results were obtained by chemical assay. The A/G ratio was remarkably constant and would indicate good protein metabolism and liver function.

The electrophoretic separations of the serum proteins (Table 5) are based on serum collected over an 8-month period. There were some rather wide fluctuations, the causes of which are unknown. (Globulin fluctuations might be the result of response to challenges by various microorganisms, but these were not evident in the WBC count.)

References

Defran, R. G. & Pryor, K. (1980). The behavior and training of cetaceans in captivity, Chap. 7, p. 321. In: *Cetacean Behavior: Mechanisms and Functions*. Herman, L. M. (ed.). John Wiley & Sons, New York, NY.

DeMonte, T. & Pilleri, G. (1971). Plasmaprotein fractions in some species of cetaceans (*Inia, Platanista, Phocoenoides, Eschrichtius*). In: *Investigations on Cetacea*, Pilleri, G. (ed.) Berne, **3,** 219–225.

DeMonte, T. & Pilleri, G. (1979*a*). Cetacean Hematology I. Hemoglobin. In: *Investigations on Cetacea*, Pilleri, G. (ed.) Vol. X, Chapter VI, 277–288.

DeMonte, T. & Pilleri, G. (1979*b*). Cetacean Hematology II. The Blood Cells. In: *Investigations on Cetacea*, Pilleri, G. (ed.) Vol. X, Chapter VI, 289–311.

DeMonte, T. & Pilleri, G. (1982). Cetacean Hematology III. Plasma Proteins. In: *Investigations on Cetacea*, Pilleri, G. (ed.) Vol. XIV, 197–229.

Evans, W. E. (ed.) (1974). The California Gray whale. Special number. *Mar. Fish. Rev.* **36(4),** 1–64.

Geraci, J. R. & Medway, W. (1973). Simulated field blood studies in the bottlenosed dolphin *Tursiops truncatus*. 2. Effects of stress on some hematologic and plasma chemical parameters. *J. Wildl. Dis.* **9,** 29–33.

Gilmartin, W. G., Pierce, R. W. & Antonelis, G. A. Jr. (1974). Some physiological parameters of the blood of the California Gray whale. *Mar. Fish. Rev.* **36(4),** 28–31.

Gilmore, R. M. (1955). The return of the Gray whale. *Scientific American*, January.

McVay, S. (1966). The last of the great whales. *Scientific American*, August.

McVay, S. (1973). Stalking the Arctic whale. *American Scientist* **61(1),** 24–37.

Medway, W. (1974). Some coagulation factors in plasma from a California Gray whale, *Eschrichtius robustus*. *Mar. Fish. Rev.* **36(4),** 24–25.

Medway, W. & Geraci, J. R. (1964). Hematology of the bottlenose dolphin (*Tursiops truncatus*). *Am. J. Physiol.* **207(6),** 1367–1370.

Medway, W., Geraci, J. R. & Klein, L. V. (1970). Hematologic response to administration of a corticosteroid in the bottle-nosed dolphin (*Tursiops truncatus*). *J.A.V.M.A.* **157(5),** 563–565.

Mitchell, E. D. (1973). The status of the world's whales. *Nature, Canada* **2(4),** 9–27.

Ridgway, S. H. (ed.) (1972). *Mammals of the Sea, Biology and Medicine*. Charles C. Thomas, Chicago.

Rudd, J. T. (1956). The Blue whale. *Scientific American*, December.

Schalm, O. W., Jain, N. C. & Carroll, E. J. (1975). *Veterinary Hematology*. 3rd edn. Lea & Febiger, Philadelphia, PA.

Sidwell, V., Fonhcannon, P. R., Moore, N. S. & Bonnet, J. C. (1974). Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish and mollusks. 1. Protein, fat, moisture, ash, carbohydrate, energy value and cholesterol. *Mar. Fish. Rev.* **36(3),** 21–35.

Spencer, M. P. (1973). Scientific studies on the Gray whales of Laguna Ojo de Liebre (Scammon's Lagoon), Baja California, Mexico. *National Geographic Society Research Reports*, 1966 projects, pp. 235–253.

Wahrenbrock, E. A., Maruschak, G. F., Elsner, R. & Kenney, D. W. (1974). Respiration and metabolism in two Baleen whale calves. *Mar. Fish. Rev.* **36(3),** 3–9.

Walker, T. J. (1971). The California Gray whale comes back. *National Geographic* **139(3),** 394–415.

Zettner, A. (1974). Some hematologic observations on the California Gray whale, *Eschrichtius robustus*. *Mar. Fish. Rev.* **36(4),** 22–24.

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Individual chlorinated biphenyls and pesticides in tissues of some cetacean species from the North Sea and the Atlantic Ocean; tissue distribution and biotransformation

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Summary

Individual chlorinated biphenyls (CBs) and organochlorine pesticides (α- and γ-HCH, dieldrin, HCB, p,p′-DDT, p,p′-DDD, and p,p′-DDE) were investigated in blubber, brain, heart, kidney, liver and muscle of 19 cetaceans from the Dutch coastal area, the open North Sea and the western Atlantic. Multidimensional gas chromatography (MDGC) with electron capture detection (ECD) was used to check the results of single capillary column GC-ECD data.

Absolute concentrations and concentration patterns of individual compounds were investigated to describe their distribution between tissues and to interpret the findings in terms of lipophilicity and persistence to biotransformation of individual compounds. A clear relation could be established between the chemical structure of each CB and its biomagnification in marine mammal tissues. Persistent congeners are biomagnified but metabolizable congeners appeared at much lower extractable lipid based concentrations than in the food sources. The CBs with the highest concentrations in the mammal tissues were hexa- and heptachloro-biphenyls.

The results are compared with published data on individual CBs in cetaceans and in blood of seals from the coastal area. CB patterns in different tissues of any organism were almost identical. A large similarity in the patterns of persistent congeners was also found between tissues and organs of mammals from widely separated oceanic areas, although absolute concentrations differed sometimes by more than an order of magnitude. Differences were found in the relative contributions of less persistent congeners. The apparent rates of biotransformation were higher in seals than in cetaceans, for which the lowest rates were generally observed in the animals from the open sea and in particular in *Physeter macrocephalus* and also *Sotalia fluviatilis*.

Especially CB congeners with vicinal H-atoms only in the m,p positions were more rapidly metabolized in seals than in cetaceans. This can be due to the lower activity of the MFO enzyme system at lower CB levels or to physiological differences between species. CB congeners with vicinal H-atoms in the o,m positions with not more than one ortho-chlorine were metabolized in each case.

Of the most toxic CB congeners, i.e., those showing a 3-MC or mixed 3-MC and PB- type MFO induction in rats, CBs -77, -105, -118, -128, and -138 were present (although at low concentration levels), but CBs -81, -114, -123, -126, -167, -157, -169, and -189 were below detection levels.

The concentrations of individual CBs were of similar magnitude as those of some pesticides. In Wadden Sea fish and in porpoises from the coastal North Sea, p,p′-DDD was the dominant contributor to ∑-DDT but in the open North Sea and the western Atlantic, p,p′-DDE was by far the largest contributor (80–90%).

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Introduction

Several organochlorines such as Polychlorinated biphenyls (PCBs) and pesticides, e.g., members of the DDT family, dieldrin, etc., are strongly lipophilic compounds, which concentrate in lipid tissues of marine organisms. There is ample evidence that 'PCBs' and 'DDTs' are at least partly responsible for reproductive and immunological abnormalities (Hook & Johnels, 1972; Fuller & Hobson, 1986; Helle, Olsson & Jensen, 1976a,b; Olsson, Johnels & Vaz, 1975; Anon., 1987) observed in some marine mammal populations. This has been reported for sea lions in Californian coastal waters (De Long *et al.*, 1973; Gilmartin *et al.*, 1976), for common seals and harbour porpoises of the Dutch Wadden Sea (Reijnders, 1978, 1979, 1986), for grey seals, ringed seals and harbour seals in the Baltic (Helle, 1980, 1981, 1986; Helle, Olsson & Jensen, 1976a,b; Almkvist, 1982) and for belugas in the St. Lawrence

river estuary (Béland, Michaud & Martineau, 1987; Martineau *et al.*, 1987).

In fish and invertebrates, equilibrium partitioning between body lipids, blood and ambient water is the dominant mechanism for elimination of apolar compounds (Hamelink, Waybrant & Ball, 1971). This mechanism, involving exchange between blood and water via the gills is not available for marine mammals. This accounts at least partially for the considerably higher concentration levels of some apolar compounds in marine mammals than in lower organisms.

The relative contributions of individual chlorinated biphenyls (CBs) to their mixtures (i.e., the CB patterns) in marine mammal tissues differ from those in lower organisms. In particular, congeners with vicinal H-atoms in the m,p positions or with vicinal H-atoms in the o,m positions in combination with not more than one ortho-chlorine, are missing in mammals or present at only minor concentration levels. This is an indication for the significant role of enzyme-mediated metabolism in the elimination of these congeners that are transformed into more polar compounds with increased water solubility. Also reactivity may be increased, (Boon *et al.*, 1987). For example, lower plasma levels of vitamin A and thyroxine in rats and mice appeared to be caused by a metabolite of CB-77, but not by the parent compound (Brouwer *et al.*, 1986). Metabolism is of vital importance for the elimination of lipophilic substances in marine mammals (Abdel-Hamid, Moore & Matthews, 1981; Gage & Holm, 1979; Sipes *et al.*, 1980).

In a recent literature review (Reuthergaardh & Knap, 1987) it was concluded that (i) information on organochlorine levels in other tissues than blubber is scarce, (ii) much less information is available for cetaceans than for seals and (iii) practically all data have been obtained with the use of packed column gaschromatographic separation techniques with technical PCB mixtures (e.g., Aroclors) as reference materials. The resulting quantitative data on 'PCBs' contain therefore very limited useful information on individual chlorinated biphenyls. As the latter have different physical chemical properties, a more accurate insight into the mechanisms of uptake, distribution between tissues and elimination can be gained from studies of individual, well defined congeners in various tissues, including the major depot tissue (blubber) as well as possible target organs.

We report here on the distribution of chlorinated biphenyl congeners and organochlorine pesticides in blubber, brain, heart, kidney, liver and muscle of 19 Cetaceans originating from the Dutch coast, the open North Sea and the open and western Atlantic. Most animals were stranded harbour porpoises (*Phocoena phocoena*), whose population in coastal North Sea waters has declined very strongly in the last decades (Verwey, 1975). The other animals were stranded open ocean animals or animals captured along the Western Atlantic coast, transferred to the Dolfinarium at Harderwijk in the Netherlands and fed with mackerel from the open Atlantic until death (when their tissues were sampled for analysis). The data will be used for comparison of absolute and relative concentrations of individual compounds between tissues and species from different regions in relation to food source and lipid physiology. Also, the present data on cetaceans are compared in detail with published data of CB congeners in harbour seals from the Dutch coastal area (Boon *et al.*, 1987).

Methods and materials

Collection of animals

Most animals described here were found dead along the Dutch coast in 1977–1979. Collection and transport of the carcasses of the protected species were authorized and controlled by two designated institutions (Zoological Museum, Amsterdam and Museum for Natural History, Leiden). Samples were taken under appropriate non-contaminating conditions, deep frozen in precleaned glass containers and transported to the Netherlands Institute for Sea Research at Texel. Only when degradation had not proceeded too far, were animals selected for analysis. Animals which had been caught alive were kept for some time at the Dolfinarium in Harderwijk. The available data for all animals is given in Table 1. Animal 15, a male sperm whale, was much larger than all other animals investigated.

Analytical Methods

After thawing of samples at room temperature, subsamples were taken, weighed and extracted with n-hexane in a Soxhlet extractor for 10 hours. Lipid content was determined as the n-hexane soluble fraction. An aliquot of the extract, containing 40-50 mg lipid was used for the analysis of organochlorines. Cleanup was performed over alumina and organochlorines were separated into classes over a silica column. Details have been described before (Duinker & Hillebrand, 1983b).

The first silica fraction contained hexachlorobenzene (HCB), p,p′-DDE and all CBs quantitatively. The second fraction contained α- and γ-hexachlorocyclohexane (HCHs), dieldrin, p,p′-DDT, p,p′-DDD and several other, yet unidentified compounds. Analysis was performed by temperature programmed capillary gas chromatography with electron capture detection (GC-ECD) on a SE-54 column.

Compounds were identified with a mixture of pure reference compounds on the basis of retention times.

Table 1. Identification and specification of all mammals discussed in the paper

Animal number and species		Sex	Length (cm)	Weight (kg)	Age (y)	Found		Status
						Location	Time	
1	*Phocoena phocoena*	f	135	29	7	53°22N, 5°15E Dutch coast	7.8.78	dead
2	*Phocoena phocoena*	f	128	27	2.5	52°42N, 4°10E Dutch coast	29.3.78	dead
3	*Phocoena phocoena*	f	151	49	—	53°15N, 4°55E Dutch coast	12.4.79	dead
4	*Phocoena phocoena*	f	93	12	—	52°8N, 4°15E North Sea	7.3.77	dead
5	*Phocoena phocoena*	f	—	58		52°50N, 4°38E Dutch coast	11.6.78	alive[a]
6	*Phocoena phocoena*	f	155	39	7.5	52°60N, 4°40E Dutch coast	14.12.78	dead
7	*Phocoena phocoena*	f	153	43	>11	53°24N, 6°25E Dutch coast	8.3.78	dead
8	*Phocoena phocoena*	f	160	—	—	51°51N, 3°58E Dutch coast	17.10.78	dead
9	*Phocoena phocoena*	f	150	—	—	53°10N, 4°50E Dutch coast	5.4.77	dead
10	*Phocoena phocoena*	m	105	—	<1	52°7N, 4°15E Dutch coast	20. 3.77	dead
11	*Phocoena phocoena*	m	105	18	—	52°22N, 4°33E Dutch coast	14. 4.77	dead
12	*Lagenorhychnus albirostris*	f	242	100	—	57°N, 1°W North Sea	4.7.77	dead
13	*Lagenorhychnus albirostris*	m	239	134	—	57°N, 1°W North Sea	4.7.77	dead
14	*Lagenorhychnus albirostris*	f	235	95	—	53°7N, 4°45E North Sea	30.9.77	dead
15	*Physeter macrocephalus*	m	1522	30 000	11	52°34N, 4°35E Dutch coast	15.12.79	dead
16	*Delphinus delphis*	f	170	—	—	53°10N, 4°50E Dutch coast	6.3.79	dead
17	*Sotalia fluviatilis*	f	170	50	8	9°25N, 75°40W San Antero, Cordoba	10.7.77	alive[b]
18	*Sotalia fluviatilis*	m	170	55	9	9°25N,75°40W San Antero, Cordoba	10.7.77	alive[c]
19	*Tursiops truncatus*	f	237	—	32	26°6N, 79°55W Off Fort Lauderdale	3.7.65	alive[d]

a–d: animals were transferred to Dolfinarium, Harderwijk, the Netherlands, where they died on 7.8.78. (a), 14.1.78 (b), 19.12.77 (c, named Ramon) and 7.1.78 (d, named Mama). Registration numbers at the Zoologisch Museum, Amsterdam (ZMA) and Rijks Museum voor Natuurlijke Historie (RMNH, Leiden) are ZMA 20013 (1), ZMA 19799 (2), ZMA 19205 (3), ZMA 19203 (4), ZMA 20164 (5), ZMA 20336 (6), ZMA 19797 (7), RMNH 27281 (8), RMNH 25754 (10), ZMA 19206 (11), Museum den Haag (15), ZMA 19780 (17) and ZMA 19775 (18).

This did not resolve the problems associated with the possibility of the presence of co-eluting compounds. It was realized that this was the case for several peaks on the basis of peak shape and it was suspected to be the case for in principle each PCB peak (Duinker & Hillebrand, 1983a). This problem has been resolved since Mullin *et al.* (1984) have published the retention properties of all 209 congeners on a SE-54 column. In addition, multidimensional gas chromatography with electron capture detection (MDGC-ECD) was introduced recently. The technique allows the unambiguous determination of individual CBs and other compounds in complex mixtures, e.g., technical PCB mixtures and seal blubber (Duinker, Schulz & Petrick, 1988a; Schulz, Petrick & Duinker, 1989). Thus, the presence of the following pesticides was recently confirmed for the present samples at the Institute for Marine Research

at Kiel: p,p′-DDT, p,p′-DDD, p,p′-DDE, HCB, dieldrin, α-HCH, and γ-HCH. The technique showed that aldrin, endrin and o,p′-DDD were not present, although the retention times on SE-54 were consistent with their presence (Fig. 1). In the remainder of this paper, p,p′-DDD, p,p′-DDT and p,p′-DDE will be indicated by DDD, DDT and DDE.

The CBs often found to be present in relatively high concentrations in the mammal tissue extracts as well separated peaks (identification in terms of numbers) are: 52, 49, 44 (tetrachloro-CBs), 101, 118 (pentachloro-CBs), 149, 153, 138 (hexachloro-CBs), 187, 183, 177, 172, 180 (heptachloro-CBs), 201, 194 (octachloro-CBs), 206 (nonachloro-CB), and 209 (decachloro-CB). The molecular structures of CBs discussed in this paper are given in Table 2.

CB-90 (2,2′,3,4′,5) was found to interfere with the determination of CB-101, but its contribution to the peak was $<10\%$. It was also shown that with the SE-54 column used for analysis, CB-153 was well separated from CBs-132 and -105 (Duinker, Schulz & Petrick, 1988b).

The congeners analyzed here contribute about 60% to the total peak area of all CBs in any of the tissues (Fig. 1). As the analysis was restricted to unambiguously determined compounds (with a small, up to 10% contribution accepted for one congener, i.e., 90/101), quantitative results and comparisons can be considered reliable, as far as chromatographic overlap is concerned.

The lipid content of a tissue or organ appears to be an essential factor to determine its overall content of lipophilic compounds on a wet or dry weight basis. Therefore, we report concentrations on an hexane-extractable lipid basis. Wet weight concentrations are inadequate to establish comparisons between different organs of the same individual and between different individuals in a population or different species, because of variations in lipid contents. If required, however, they can be readily obtained from lipid based concentrations and the appropriate lipid contents given for each tissue and organ in Table 3.

The weight concentrations on a lipid basis (e.g., $\mu g g^{-1}$ lipid) of 7 pesticides and 17 individual chlorobiphenyls (CBs) in 87 tissues of 19 animals, which are the basic results, have been transformed into molar concentrations. Absolute concentrations are expressed as nmol (or pmol) per gram lipid. They are useful for comparing concentration levels and expressing biomagnification factors.

Representation of the compositions of CB- and pesticide mixtures

Different compositions of mixtures may occur in different tissues as result of differences in kinetics and/or mechanisms of distribution. These effects will be most easily observed from a study of the relative concentration values of the contaminant mixtures, irrespective of actual concentration levels (Duinker *et al.*, 1988). The relative concentrations of CBs have been calculated as mol % for each of the compounds in the appropriate mixture of CBs, and similarly so for the pesticides. e.g., as mol % of the total concentration. The effect of differences in absolute concentrations between samples is eliminated in this way.

The mol % contribution $F_k^{t_i,a_j}(\%)$ of a chlorinated biphenyl congener k, (CB_k, where $k=1 \ldots 17$) in

Table 2. Structure and molecular weights (M) of pesticides and chlorinated biphenyls discussed in this paper. The order in which the CBs appear in the list is the same as in the Figures and Tables (reflecting the elution pattern from a SE-54 column)

	M	Structure
Pesticide		
α-HCH	288	α-isomer of hexachlorocyclohexane
γ-HCH	288	γ-isomer of hexachlorocyclohexane
dieldrin	378	3,4,5,6,9,9-hexachloro-1a, 2, 2a, 3, 6, 6a, 7, 7a octahydro-2.7:3,6 dimethano naphth-[2,3-b] oxirene dimethanonaphthalene
HCB	282	hexachlorobenzene
p,p′-DDD	318	2,2-bis(p-chlorobiphenyl)-1, 1-dichloroethane
p,p′-DDT	352	2,2-bis(p-chlorobiphenyl)-1, 1-trichloroethane
p,p′-DDE	316	2,2-bis(p-chlorobiphenyl)-1, 1-dichloroethene
Chlorinated biphenyls		
18	258	2,2′,5
26	258	2,3′,5
52	292	2,2′,5,5′
49	292	2,2′,4,5′
44	292	2,2′,3,5′
70	292	2,3′,4′,5
84	326	2,2′,3,3′,6
101	326	2,2′,4,5,5′
99	326	2,2′,4,4′,5
136	361	2,2′,3,3′,6,6′
107	326	2,3,3′,4′,5
149	361	2,2′,3,4′,5′,6
118	326	2,3′,4,4′,5
153	361	2,2′,4,4′,5,5′
138	361	2,2′,3,4,4′,5′
187	395	2,2′,3,4′,5,5′,6
183	395	2,2′,3,4,4′,5′,6
128	361	2,2′,3,3′,4,4′
177	395	2,2′,3,3′,4′,5,6
172	395	2,2′,3,3′,4,5,5′
180	395	2,2′,3,4,4′,5,5′
201	430	2,2′,3,3′,4′,5,5′,6
194	430	2,2′,3,3′,4,4′,5,5′
206	464	2,2′,3,3′,4,4′,5,5′,6
209	498	2,2′,3,3′,4,4′,5,5′,6,6′

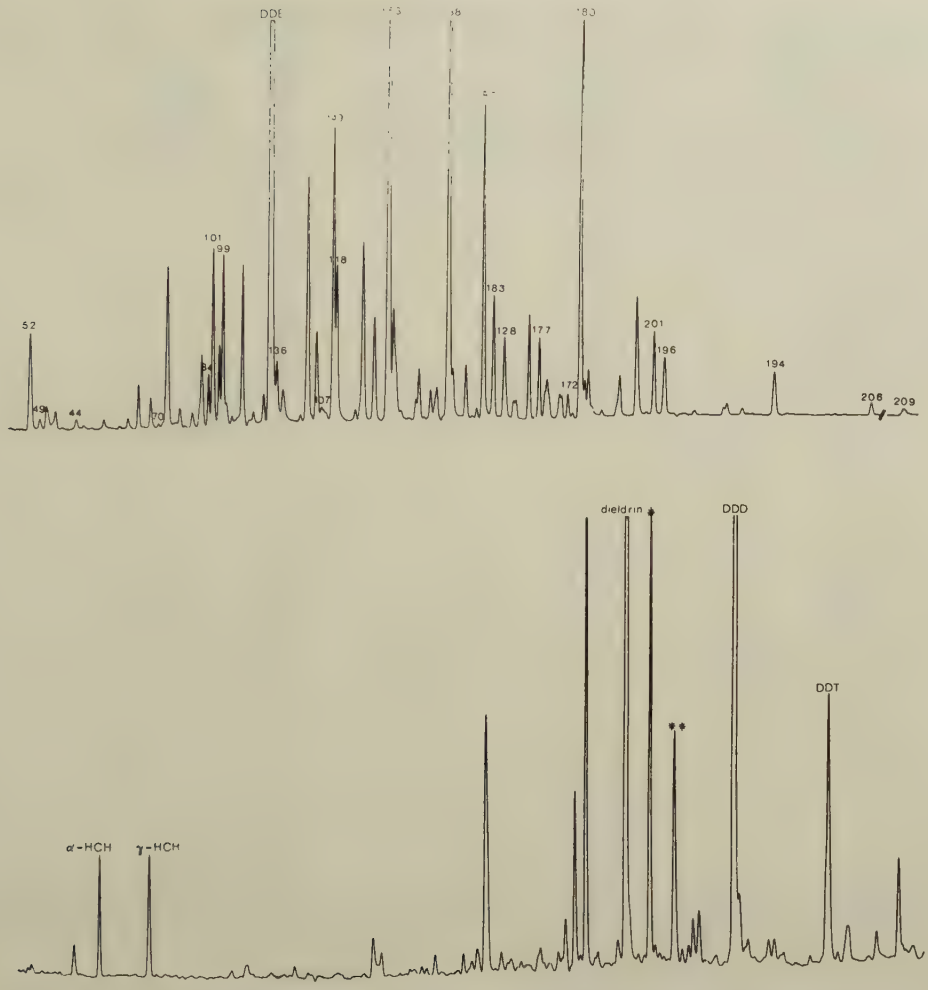

Figure 1. ECD-chromatograms of the PCB- (i.e., the first silica gel) fraction of heart of animal 16 (top) and the pesticide- (second silica) fraction of muscle of animal 6 (animals are described in Table 1).

Top: CB congeners have been identified by numbers (Table 2) according to Ballschmiter and Zell (1980). DDE = p,p′-DDE. Non-identified congeners were not analysed because of overlap problems (Duinker, Schulz & Petrick, 1988a). The strong peak of hexachlorobenzene (HCB) and the extremely weak peaks of CB-18 and CB-26 appear early in the chromatogram (not depicted here).

Bottom: Pesticides have been identified at the apex of the peaks (DDD = p,p′-DDD, DDT = p,p′-DDT). The positions where endrin and o,p′-DDD elute have been indicated by * and **, respectively. For chromatographic conditions see Duinker & Hillebrand, 1983b.

tissue i (t_i where i = 1 ... 6 representing blubber, brain, heart, kidney, liver and muscle) of animal j (a_j where j = 1 ... 19) was calculated from

$$F_k^{t_i,a_j}\% = \frac{(CB_k)^{t_i,a_j}}{\sum_{r=1}^{17} (CB_r)^{t_i,a_j}} \times 100$$

Parentheses are used to indicate molar concentrations. Calculations for the pesticides were carried out analogously. Average mol % contributions were calculated for each tissue type (e.g., blubber) in the two groups of animals.

Results

Distinction of groups of animals and groups of CB congeners

We have treated the harbour porpoises (animals 1–11) and the other animals (12–19) as two separate

Table 3. Hexane extractable lipid contents (% of wet weight) in the various tissues. Averages ($\bar{x}$), number (n) of data included in the statistics, relative standard deviation (s %) and range of values are given in the bottom rows. Data for *Macrocephalus* (animal 15) not included in statistics. —: tissue not analysed

Animal number	Lipid percentage in						
	Blubber	Brain	Heart	Kidney	Liver	Muscle	Sperm
1	92.9	8.2	1.5	1.8	2.3	0.5	—
2	82.0	7.5	1.7	2.5	4.1	7.9	—
3	77.8	11.2	—	1.7	8.6	0.8	—
4	67.7	5.3	—	1.7	2.3	0.7	—
5	90.6	6.5	—	—	—	2.7	—
6	93.5	10.6	2.1	6.7	2.1	0.8	—
7	88.2	13.9	1.8	2.3	3.3	1.0	—
8	—	—	—	—	3.9	—	—
9	90.2	9.4	—	2.7	2.4	—	—
10	54.2	7.2	—	1.4	6.0	1.5	—
11	72.4	6.6	—	1.9	3.2	1.0	—
12	91.7	9.5	—	—	—	1.2	—
13	79.8	8.1	—	—	—	1.1	—
14	97.9	8.3	—	—	—	0.8	—
15	92.1	18.8	18.9	—	—	18.5	97.0
16	93.9	8.7	3.5	6.0	2.5	1.2	—
17	76.2	8.3	3.5	2.1	6.7	1.5	—
18	90.8	7.4	3.2	5.0	9.6	4.1	—
19	96.1	13.4	—	—	—	2.0	—
$\bar{x}$	84.5	8.8	2.5	3.0	4.4	1.8	—
range	54.2–97.9	5.3–13.9	1.5–3.5	1.4–6.7	2.1–9.6	0.5–7.9	—
s (%)	13.5	25.6	33.0	59.4	55.2	100.6	—
n	17	17	7	12	13	16	1

groups. Harbour porpoises (group A) dwell in the coastal regions of the North Sea and represent a single species, while all other species (group B) live in the open North Sea or the Atlantic Ocean. Group B is rather heterogeneous; for each species either one, two or three animals were available. This did not justify a further subdivision of this group. The main argument to consider these animals, tentatively, as a group is the similarity of the composition of the pesticide mixtures in all tissues of these animals; their compositions differ considerably from those in the porpoise tissues (Fig. 2). Statistical analyses of the results, to be discussed below, justify this procedure for the pesticides. Differences in relative concentrations of some congeners between different species within group B could also be observed within group B (Fig. 3).

The CB congeners are divided into groups I and II, involving the congeners that were apparently metabolized (group I) and those that were persistent in harbour seals (group II, Boon *et al.*, 1987).

Most animals were females. Because of the limited number of animals investigated, differences in organochlorine concentrations due to differences in sex (Addison & Brodie, 1977; Tanabe *et al.*, 1981a,b, 1982; Duinker & Hillebrand, 1979b; Alzieu, Duguy & Babin, 1982) and age (Norstrom *et al.*, 1988; Muir *et al.*, 188; Reijnders, 1979; Addison & Smith, 1974) were not recognized.

Lipid based concentrations

The mean lipid based concentrations of CBs and pesticides in harbour porpoises (group A) and the combined 'open sea species' (group B) are given in Tables 4 and 5 together with the matching standard deviations and the quotient of the mean concentrations of groups A and B for each compound. For statistical comparisons of the means of animal groups A and B, a Student's *t*-test was used when the samples were not significantly inhomogeneous (i.e., $p > 0{\cdot}05$), as determined with an F_{max} test with seven degrees of freedom. Often the variances of the samples were heteroscedastic, however, in which case an approximate *t*-test was used (Sokal and Rohlf, 1969).

Averages are depicted in Figures 4 and 5, including graphs for the tissues of one individual animal for each group for comparison with average values. It shows to what extent the data of an arbitrarily selected individual animal reproduces the average values for both groups A and B.

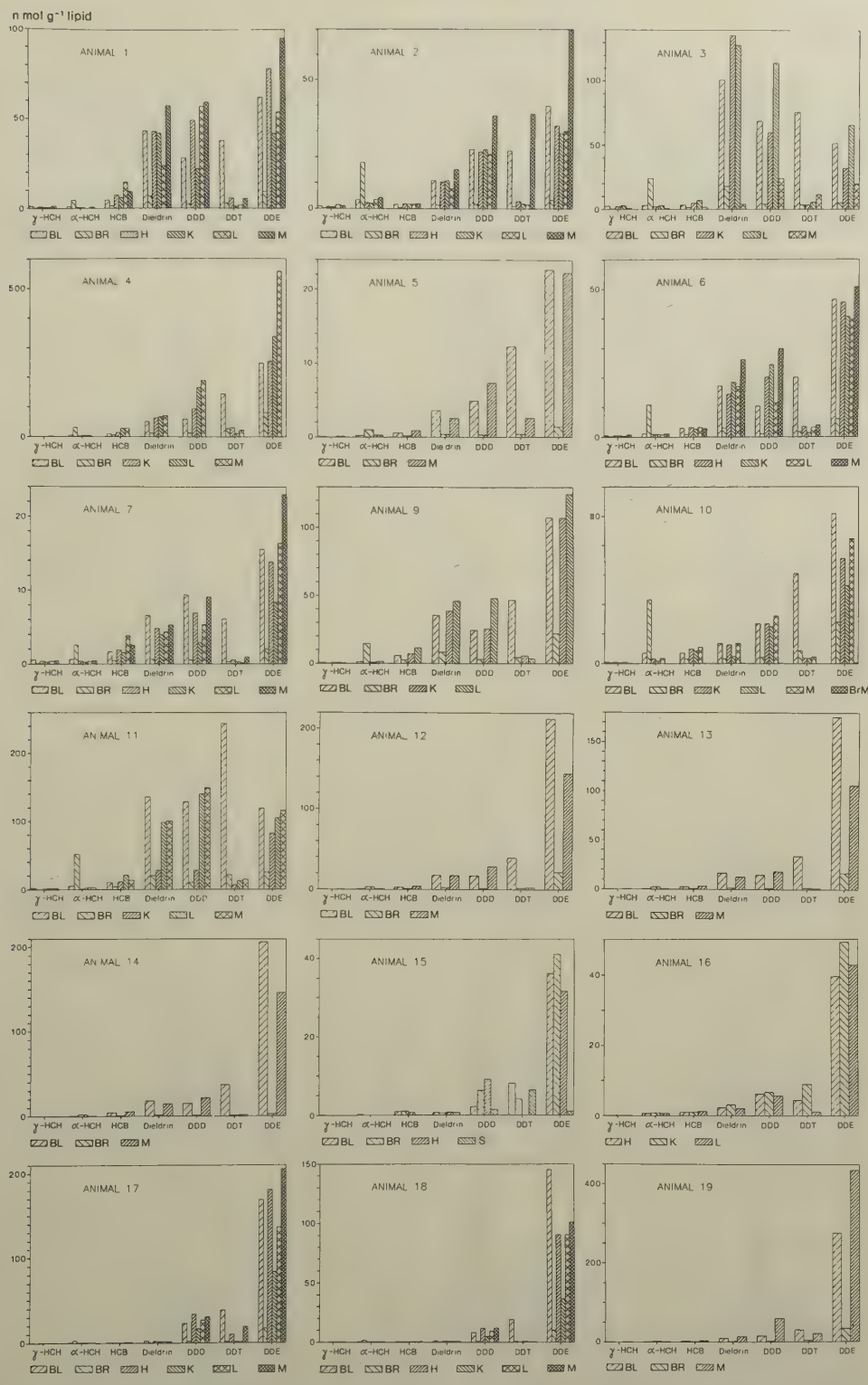

Figure 2. Concentrations (nmol g^{-1} hexane extractable lipid) of γ-HCH, α-HCH, HCB, Dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p,p′-DDE (DDE) for each tissue analysed in all individual animals 1–19. Animals 1–11 are porpoises (group A) and animals 12–19 represent other species (group B), see Table 1. The y axes have different scales. The data for animal 8 (one tissue analysed) appear in other Figures.

BL = blubber, BR = brain, K = kidney, L = liver, M = muscle, S = sperm (animal 15).

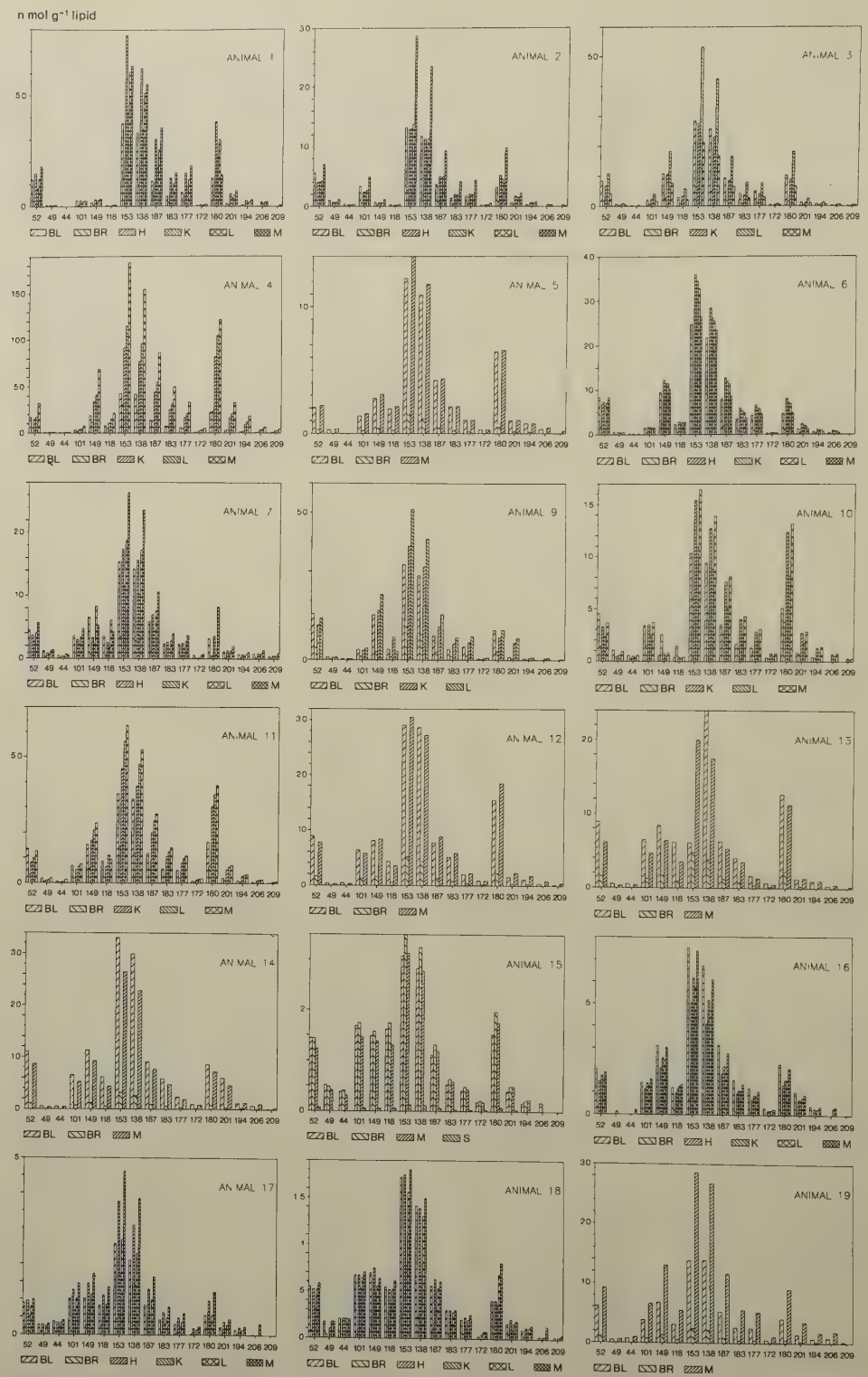

Figure 3. Concentrations (nmol g $^{-1}$ hexane extractable lipid) of CBs for each tissue analysed in all individual animals 1–19. Animal and tissue identifications as in Figures 1 and 2. The y axes have different scales. The data for animal 8 (one tissue analysed) appear in other Figures.

Most data in the literature have been presented in terms of w/w (e.g., ng g^{-1} lipid or wet weight). Also, most concentrations have been given in terms of commercial mixture equivalents, such as Aroclor 1260. In order to allow—admittedly crude—comparisons with such literature data, concentrations of individual CBs (ng g^{-1} extractable lipid) have been totalled for each tissue and presented in Table 6.

Chlorinated biphenyls

Comparison of concentrations between groups A and B

The average lipid based concentrations of each CB congener are higher in the harbour porpoises (group A, Table 4) than in the 'open sea species' (group B, Table 4), but particularly as a result of the large standard deviations within both groups these differences are not always significant (i.e. p not always <0.05). The ratios of the average absolute concentrations in group A/group B are given in Table 4, together with the matching significance levels. For statistical comparisons between the same tissue type of the animal groups A (harbour porpoises) and B (combined open sea species) an F_{max} test was performed prior to significance testing to establish whether the data to be compared had homogeneous variances. If $s^2_{max}/s^2_{min} > F_{.05[2.7]}$, a normal Student's *t*-test was used; else an adapted *t*-test for comparison of two heteroscedastic groups of samples was used (Sokal and Rohlf, 1969). The largest differences in concentrations between groups A and B are present in heart, kidney and liver, while the concentrations of only a few CB-congeners are significantly different in blubber, brain and muscle. CB-44 never differed significantly between both groups of cetaceans.

Concentration ratios within groups A and B

The ratios of lipid based concentrations of any compound X in different tissues of the same animal can be used to compare relative lipid affinities of X. For each compound, blubber/brain, blubber/heart, blubber/kidney, blubber/liver and blubber/muscle ratios have been calculated. Average ratios ± s.dev. for each tissue of groups A and B are presented in Tables 7 and 8. Average ratios are depicted in Figures 6 and 7, including ratios for an individual animal from each group for comparison with the average values. The figures reflect the fact that in each animal, concentrations of all CBs are similar in heart, kidney, liver and muscle. They are considerably lower in brain, however, on average 4 times lower in harbour porpoises and 6–7 times lower in group B animals.

The concentration of each CB in blubber differs from those in heart, kidney, liver and muscle. It is interesting in this respect that in harbour porpoise samples the average concentration ratio blubber/X (X representing heart (h), kidney (k), liver (l), and muscle (m)), decreases from around 1.0 (for the tetrachloro-CB 52) to values around 0.2–0.4 (for nonachloro-CB 206) (Table 7 and Fig. 6). In the group B species, average bl/br ratios are much higher (4–8). The bl/X ratios for the other tissues in these animals are around 1, but distinctly different among them (bl/h 0.9, bl/l 1.1, bl/m 0.9, bl/k 1.5). In these cases, the exceptional values of CB-209 have been deleted. In contrast to the situation in the harbour porpoises, they remain constant over the range of congeners (Table 7, Fig. 6). These differences between blubber and the other tissues are not obvious from visual inspection of the CB patterns. In the following, we shall consider the patterns in blubber and the other tissues as similar, realizing that there are the differences mentioned above. We feel that more data are required to establish whether the observed effects are real and may have wider validity.

Pesticides

Comparison of concentrations between groups A and B

The lipid based concentrations of the pesticides α- and γ-HCH, HCB and dieldrin are higher in most tissues of the harbour porpoise compared to the open sea species (Table 5). In contrast, the concentrations of p,p′-DDE were higher in the open sea animals. Differences of p,p′-DDD and p,p′-DDD and p,p′-DDT are small compared to the other pesticides and are not significant.

Concentration ratios within groups A and B

The differences in concentrations in blubber relative to brain are larger for pesticides than for CBs. Average bl/br ratios increase in the series HCB (3), dieldrin and DDE (6–8), DDD (12), DDT (16). In group B animals, even higher average ratios occur for DDE (19) and DDT (24). α-HCH, however, had a high concentration in brain lipids: average bl/br ratios are considerably smaller than 1.0 (about 0.2 in both groups). The large relative concentration of DDT in blubber is obvious for both groups of animals (bl/X ratios between 8 and 20 in animals of group A and between 20–60 in animals of group B). Much smaller differences in concentration ratios were found for the other compounds and tissue types (thus, excluding α-HCH, DDT and brain).

Compositions of CB- and pesticide mixtures

Chlorinated biphenyl patterns

Distribution between various tissues of the same animal

The compositions of CB-mixtures (whereby each concentration is expressed as mol % of the sum of all concentrations) are much more similar between different tissues of the same animal than absolute concentrations (compare Tables 4 and 9). So, the concentrations of many CB congeners covary

Table 4. Average concentrations (avg) of CBs (in nmol g^{-1} lipid) and relative standard deviation (% of average) in the various tissues of animals 1 11 (group A) and 12–19 (group B). For numbering of animals see Table 1 and for numbering of CBs see Table 2. No entry: data do not allow a reliable quantitation. Significance levels for differences between groups A and B (two-tailed *t*-test) *$p<0.05$; **$p<0.01$; ***$p<0.001$. Group I and II congeners are separated by an empty row.

Tissue	Blubber				Brain				Heart			
Animal group	A		B		A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
52	9.2	53	5.0	79	2.2	69	0.9	64	7.4	60	1.0	39
49	0.8	49	0.4	51	0.1	98	0.1	213	0.7	22	0.1	141
44	0.3	58	0.4	50	0.1	137	0.1	207	0.2	68	0.2	77
101	3.1	46	3.5	70	0.9	60	0.7	77	2.5	21	1.0	24
149	8.2	78	5.1	73	2.9	111	1.0	61	4.8	91	1.4	38
118	3.2	88	3.0	72	1.1	117	0.6	83	1.4	87	0.9	28
153	25.2	44	12.1	95	9.4	85	2.5	70	36.0	71	3.5	38
138	23.1	46	13.7	83	7.4	87	2.1	70	29.5	68	2.9	39
187	8.0	43	4.2	73	3.4	105	0.7	64	13.8	72	1.3	39
183	3.6	48	2.5	81	1.6	122	0.4	66	5.8	73	0.6	42
177	3.7	49	1.3	65	1.4	95	0.2	62	6.5	79	0.5	41
172	0.4	37	0.4	80	0.1	142	0.1	74	0.3	56	0.1	70
180	9.4	63	5.7	95	4.5	143	1.0	89	12.9	114	0.9	42
201	1.3	41	1.5	118	0.7	125	0.2	68	2.7	62	0.4	46
194	0.7	41	0.6	71	0.3	124	0.1	96	1.2	66	0.2	33
206	0.3	82	0.3	114	0.1	193	0.0	170	0.9	74	0.0	
209	0.1	154	0.0	138	0.1	192	0.0	185	0.1	173	0.0	

	Kidney				Liver				Muscle			
	A		B		A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
52	7.3	51	0.9	74	8.8	51	0.9	44	10.4	85	4.6	76
49	0.6	34	0.1	72	0.7	36	0.1	73	0.9	54	0.3	55
44	0.2	79	0.1	70	0.3	72	0.2	77	0.4	101	0.4	54
101	2.9	38	0.9	44	3.4	39	1.0	26	4.0	58	3.4	64
149	10.5	98	1.3	73	13.8	85	1.4	57	13.7	148	5.5	76
118	3.7	96	0.7	49	5.0	92	0.9	35	4.8	133	2.7	61
153	35.5	65	2.9	81	45.1	64	3.3	52	49.2	102	15.3	75
138	29.4	66	2.4	82	37.4	64	2.8	51	41.7	102	13.6	77
187	14.4	80	1.0	82	18.3	76	1.2	55	21.8	114	4.9	76
183	7.2	95	0.5	79	8.9	92	0.6	53	11.0	129	2.8	77
177	6.1	71	0.4	83	7.6	70	0.5	56	9.1	108	1.6	93
172	0.7	99	0.1	102	0.9	98	0.1	36	1.2	122	0.5	70
180	19.9	118	0.8	75	23.3	125	0.9	48	24.8	143	6.5	90
201	4.4	104	0.3	77	5.3	105	0.4	63	6.3	150	1.7	88
194	2.0	127	0.1	57	2.5	129	0.2	40	3.3	160	0.8	76
206	0.9	82	0.0	141	1.3	83	0.1	127	1.4	123	0.6	91
209	0.4	135	0.0	141	0.5	116	0.0	141	0.8	152	0.1	130

between organs and tissues of the same animal (Fig. 8). Also, a large similarity among all porpoises and among all open sea animals is observed (Fig. 8; Table 9). The differences between blubber and the other tissues was mentioned in the section titled 'Chlorinated biphenyls'.

Table 4. Continued

CBs	Ratio of averages A/B					
	Blubber	Brain	Heart	Kidney	Liver	Muscle
52	1.8	2.4*	7.6***	8.5***	9.4***	2.3
49	2.4**	1.9	7.1***	7.2***	5.4***	3.0**
44	0.8	0.8	0.9	1.5	1.5	1.1
101	0.9	1.2	2.4***	3.3***	3.5***	1.2
149	1.6	2.9	3.4*	8.4*	10.1**	2.5
118	1.1	1.9	1.7	5.3*	5.6*	1.8
153	2.1*	3.7*	10.2**	12.3***	13.6***	3.2
138	1.7	3.5*	10.3**	12.2**	13.5***	3.1
187	1.9*	4.6*	11.0**	14.3**	15.1**	4.4
183	1.4	3.8	9.2**	13.9**	14.8**	3.9
177	2.8**	5.6*	13.8**	15.5**	16.7**	5.7*
172	1.0	1.7	2.9**	8.6*	6.4*	2.4
180	1.7	4.5	14.3*	26.2*	24.9*	3.8
201	0.8	3.4	6.5*	14.5*	13.3*	3.7
194	1.1	3.6	6.1**	14.6*	13.8*	4.0
206	1.0	2.3		131.3**	11.6**	2.4
209	1.7	7.7		63.0*	76.2*	6.7

Comparison of peak patterns between groups A and B

Differences exist between the groups of animals: the ratio of the mean mol % contributions to $\sum$CB (i.e., A/B) of the potentially metabolizable congeners of group I in the groups of animals A and B is virtually always <1, but for the persistent congeners of group II this ratio is often >1. However, these differences are generally significant for only a few congeners (<1: CBs -44, -101, -118; >1: CBs -153, -138, -187, -177). Significance testing was performed in the same way as for comparison of the absolute CB concentrations (compare 'Chlorinated biphenyls'). CB-172 behaved in an unexpected way in that it belongs to group II (persistent congeners), but its ratio A/B was also often significantly <1 (Table 9).

In order to investigate in more detail whether not only the absolute concentrations but also the relative concentrations of chlorobiphenyls in the tissues of animals differ between groups A and B, a two-way analysis of variance was carried out. Concentrations were transformed into concentration ratios relative to CB-153, i.e., X/153. This enables the concentration patterns to be compared, taking into account both potential effects, viz., differences between the groups A and B and differences between the organs within each group. The underlying significance level was set at $p=0.05$.

The combined (main) effect (where p values <0.05 indicate significant differences) indicates that significant overall differences exist for several CBs (Table 10). Comparison with the values for the effects of the two groups (A and B) and the organs, indicates that the combined effects arise mainly because of differences between the groups A and B, and not because of differences between tissues within each group. The difference for CB-206 ($p<\alpha$ in the overall effect) is caused by differences between the organs and not between the animal groups. It is also observed that differences in average values were not significant for several other CBs. Most of these cases correspond to non-significant differences between the groups as well as between the organs. In conclusion, significant differences in peak patterns of CBs are present between groups A and B, taking into account the organs. Another conclusion is that biotransformation is stronger in porpoises than in the open sea species.

Pesticides

Distribution between various tissues of the same animal

A large similarity is observed between the mol % contributions of the pesticides in blubber of groups A and B (Table 5). The same applies to each of the other tissues for both groups of animals. In contrast with the situation for the CBs, the patterns for the pesticides differ significantly between the tissues, however. Despite considerable concentration differences between tissues of the harbour porpoises (Table 5), a reasonable similarity is observed for the mol % contributions of γ-HCH, dieldrin and DDE (Fig. 9). Differences are larger for α-HCH, HCB, DDD and DDT. Contributions of DDD in blubber are smaller than in liver but those of DDT are higher. The large

Table 5. Average (avg) concentrations (nmol g $^{-1}$ hexane extractable lipid) ± % standard deviation (std%) for each of the pesticides in different tissue types of the porpoises (group A) and of the other species (group B). For numbering of animals see Table 1. Significance levels for differences between groups A and B (two-tailed t-test) *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Animal group	Blubber				Brain				Heart			
	A		B		A		B		A		B	
	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
gamma-HCH	0.8	74	0.2	25	0.1	72	0.0	245	0.5	24	0.1	71
alpha-HCH	2.4	73	0.4	29	17.8	81	1.8	47	1.0	76	0.4	68
HCB	4.3	71	1.8	72	1.8	86	0.6	60	3.6	62	0.6	34
Dieldrin	41.1	103	9.3	81	7.2	96	1.0	65	18.2	81	1.4	49
DDD	36.9	99	13.5	46	3.8	106	1.9	98	24.6	62	15.1	72
DDT	63.9	111	29.3	37	6.5	133	1.8	64	3.1	57	3.6	109
DDE	76.4	86	174.5	39	16.9	135	20.3	60	42.4	55	85.9	69

Animal group	Kidney				Liver				Muscle			
	A		B		A		B		A		B	
	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
gamma-HCH	0.6	89	0.1	13	0.8	103	0.1	27	0.7	62	0.2	28
alpha-HCH	1.1	62	0.4	58	1.6	83	0.4	32	1.6	91	0.4	17
HCB	5.8	67	0.5	64	9.9	84	0.7	54	6.6	123	2.6	67
Dieldrin	38.5	101	1.7	62	40.1	105	1.3	43	28.9	115	10.1	63
DDD	32.5	80	9.3	56	58.7	97	13.8	66	35.2	117	28.2	54
DDT	5.8	158	3.6	105	4.0	100	0.7	46	9.9	112	7.3	117
DDE	70.1	101	56.4	36	83.3	109	89.7	43	99.6	157	189.6	61

	Ratio of averages A/B					
	Blubber	Brain	Heart	Kidney	Liver	Muscle
gamma-HCH	5.0**	27.3**	5.0***	4.6*	7.5*	4.4**
alpha-HCH	6.2**	9.8**	2.8*	2.6*	4.2*	4.1*
HCB	2.4*	3.0*	5.8**	12.1**	15.2**	2.5
Dieldrin	4.4*	7.1*	12.9**	22.8*	30.5*	2.9
DDD	2.7	2.0	1.6	3.5*	4.3*	1.2
DDT	2.2	3.6	0.9	1.6	5.9*	1.4
DDE	0.4**	0.8	0.5	1.2	0.9	0.5

mol % contributions of α-HCH in brain, of DDT in blubber and DDE in all tissues are striking features.

The relative concentrations of the 'DDTs' in porpoises are DDD < DDT < DDE in blubber and brain and DDT < < DDD < DDE in the other tissues (Fig. 9). These patterns are different in the open sea species (group B): considerably smaller mol % contributions occur for α-HCH in brain and very large (up to 80 mol % contributions) for DDE in all tissues (Fig. 9). The relative concentrations of DDD and DDT in blubber and liver are similar to those in the porpoises. In group B animals the order is the same as in group A but relative concentrations are different: DDD < DDT < < DDE in blubber and brain and DDT < DDD < < DDE in the other tissues.

Comparison of peak patterns between groups A and B

The same statistical approach (two-way analysis of variance) applied to the concentration ratios of CBs in different tissues of both groups A and B was also applied to the pesticides, in order to detect any significant differences between the groups A and B, taking into account the various organs. For all pesticides, significant differences in the concentration ratios X/DDE were found (Table 10). As for the CBs, the differences result mainly from differences

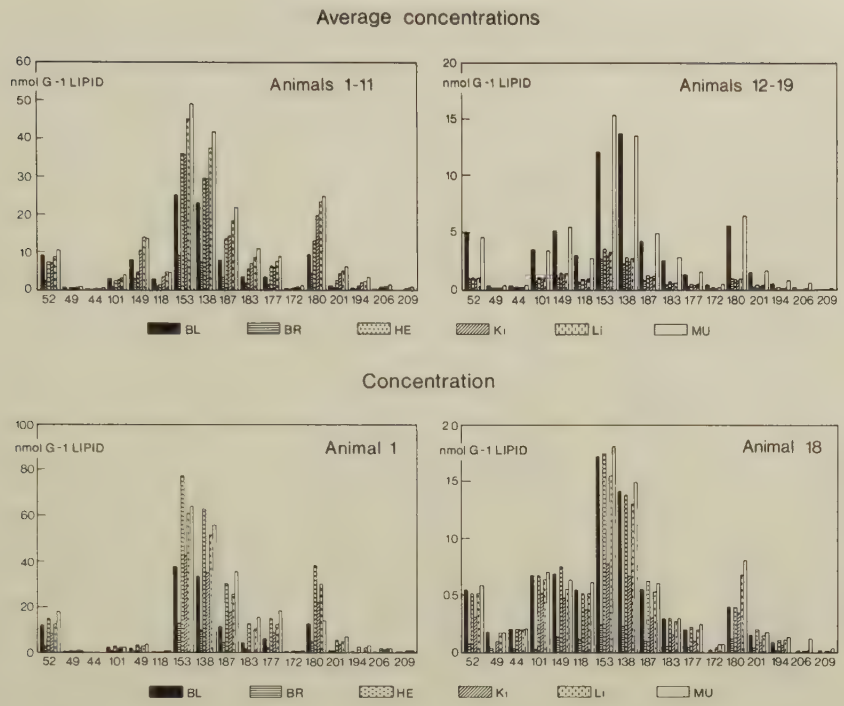

Figure 4. Concentrations (nmol g^{-1} hexane extractable lipid) of CB congeners (identified by numbers, Table 2) in blubber (BL), brain (BR), heart (HE), kidney (KI), liver (LI) and muscle (MU) of animals 1–11 (group A, left) and animals 12–19 (group B, right). The y-axes have different scales. Top: average values for groups A and B. Bottom: values for individual animals 1 and 18.

between the groups A and B and not from differences between the organs.

Discussion

Chlorobiphenyls

Chlorobiphenyl patterns in relation to metabolism

In marine mammals, food is the dominant source of contaminants, direct uptake from water being insignificant because marine mammals do not drink seawater. Blood probably plays an important role as transport compartment between different tissues (Harding & Addison, 1986). It is recognized that several congeners (hexa- and heptachloro-congeners, etc.) are persistent in the environment. CB-153 is a well known example. As it occurs with the highest concentrations of all CBs in mammal tissues, the X/153 concentration ratio is a convenient measure to estimate the effect of metabolism on the fate of X. This facilitates the comparison with the data of Boon *et al.* (1987). These authors studied the kinetics of CBs in female harbour seals. One group of animals (originating from the Dutch Wadden Sea and the Wash) was fed with Atlantic fish (mackerel) and the other group with Wadden Sea fish (plaice). CB patterns were studied in food, faeces and blood as a function of time. Highly similar patterns were found for each compartment, but patterns differed among them. Since no differences were found in CB patterns between different tissues in the present study, we assume that the CB pattern in seal blood represented the pattern in the whole seal.

Assuming the necessity of the formation of an arene oxide in the biotransformation of CBs (Safe 1984; Safe *et al.*, 1985), the differences in CB patterns between seal blood and fish most likely resulted from enzymatic bio-transformation of CBs possessing vicinal H-atoms in the m,p position or in the o,m position in combination with not more than one ortho-chlorine. In the following we make use of the data in blood and faeces of seals measured during the experiments of Boon *et al.* (1987) and of the data in tissues of cetaceans reported here. We used the original chromatograms of seal blood to calculate data on CBs which were not reported by the latter authors. As they did not measure CB-206, we have deleted this congener from the Figures and Tables that will follow.

The graphs representing X/153 concentration ratios in the paper of Boon *et al.* (1987) result in the following observations:

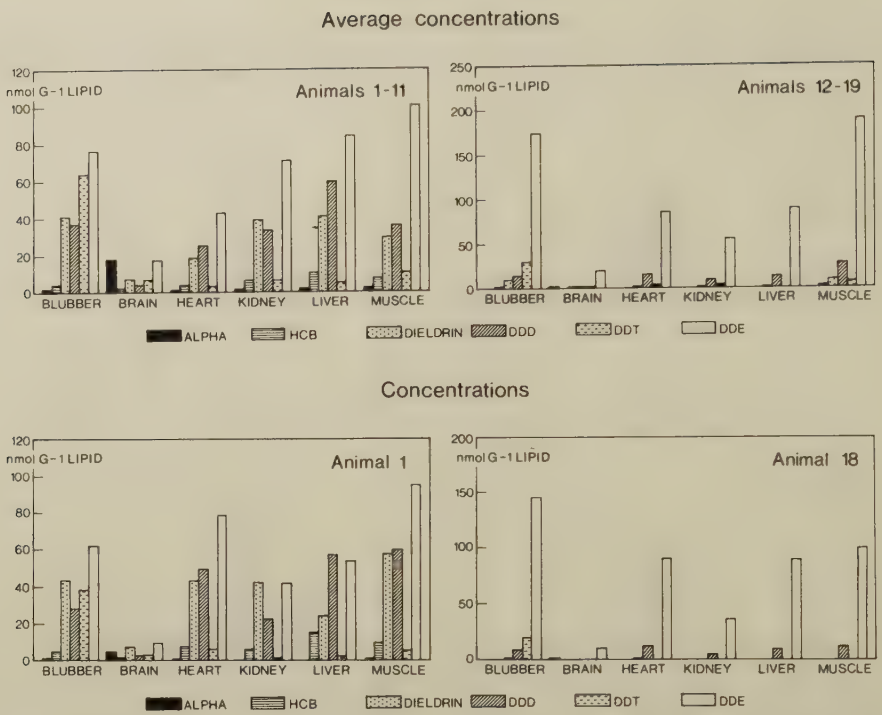

Figure 5. Concentrations (nmol g^{-1} hexane extractable lipid) of α-HCH (ALPHA), HCB, dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p,p′-DDE (DDE) in blubber, brain, heart, kidney, liver and muscle of animals 1–11 (group A, left) and animals 12–19 (group B, right). The small values of γ-HCH have not been included. The y-axes have different scales. Top: average values for groups A and B. Bottom: values for individual animals 1 and 18.

(i) the CB patterns in Wadden Sea fish (plaice) and Atlantic fish (mackerel) are very similar, although concentration levels differ by an order of magnitude.
(ii) the same CB pattern is found in faeces of animals fed with Wadden Sea or Atlantic fish.
(iii) the CB pattern in seal blood remained unchanged after feeding with mackerel for two years while the concentrations decreased by 50% in the same period.

According to the model of Boon *et al.* (1987) the following CB congeners reported here, should be metabolizable in seals: 18, 26, 44, 49, 52, 70, 84, 92, 101, 107, 118, 136 and 149. According to the model presented by Tanabe *et al.* (1988), only congeners with vicinal H-atoms in the o,m position with less than two ortho chlorines are metabolizable in cetaceans. This excludes all congeners except CBs -26, -107 and -118 from the list mentioned above for seals. CBs -99, -128, -138, -153, -172, -177, -180, -183, -187, -194, -201, -206 and -209 are persistent to enzymatic metabolism in both models.

Figure 10 shows the PCB patterns normalized to CB-153 in fish from the Dutch Wadden Sea, blood of seals fed with this fish, harbour porpoise blubber and blubber of the open sea cetaceans. In the following, seal blood and cetacean blubber are assumed to represent the situation in the entire animal. According to Table 9, this appears to be legitimate in cetaceans. The relative concentrations of the major persistent congeners resemble each other closely or are higher in mammals than in fish. Decreased relative concentrations are observed for CB-172 in porpoise blubber and CB-209 in both groups of cetaceans. When the relative concentration to CB-153 of a given congener is considerably lower in a marine mammal tissue than in fish, this indicates metabolism of that congener. This is observed for all potentially metabolizable congeners in seals, but not for CBs-52, -136, and -149 in both groups of cetaceans and CBs -84 and -101 in the cetaceans from the open sea. So, the order of capacity for biotransformation decreases in the order seals > harbour porpoises > open sea cetaceans > fish.

In the cetaceans, the CBs present in lowered relative concentrations compared to fish include all congeners according to the model of Tanabe *et al.* (1988), but also CBs -44 and -49 in both groups of cetaceans and CBs -101 and -84 in the harbour

Table 6. Sum of concentrations of 17 individual CBs discussed in this paper in tissues of porpoises (animals 1–11 group A) and the other species (animals 12–19 group B). For numbering of animals see Table 1 and of CBs see Table 2. Concentrations in $\mu g g^{-1}$ lipid. No entry: tissue not analysed.

Animal number	Blubber	Brain	Heart	Kidney	Liver	Muscle
1	126	40	269	151	219	241
2	47	9	49	48	49	99
3	110	22		101	191	74
4	186	132		434	543	825
5	48	5				52
6	91	20	126	118	114	97
7	67	12	63	45	77	104
8					155	
9	128	38		148	187	
10	43	28		70	48	75
11	158	58		201	248	277
12	120	20				125
13	89	19				84
14	134	12				107
15	17	20				17
16	32	3	21	25	24	31
17	12	2	16	8	12	20
18	8	5	8	5	8	9
19	66	11				131

porpoises (group A). All these congeners except CB-101 do contain vicinal H-atoms in the o,m position, but in combination with more than one ortho-Cl leading to persistence to biotransformation in the models of Boon *et al.* (1987) as well as of Tanabe *et al.* (1988). The apparent persistence of CBs -99, -128, -138 and -177 support the hypothesis that for the occurrence of biotransformation in the o,m region a maximum of one ortho-Cl is allowed. However, CBs -44, -49, -84, and -101 all contain vicinal H-atoms in the m,p positions. Therefore some biotransformation activity with respect to the m,p region of PCBs cannot be excluded for cetaceans in general and more specifically for *P. phocoena*, but it is certainly lower than for harbour seals. The highest relative concentrations of potentially metabolizable CBs are found in Physeter macrocephalus (animal 15, Fig. 3) and Sotalia fluviatilis (animals 17 and 18). These animals possessed the lowest absolute lipid based CB concentrations of all animals analysed (Table 6). This may reflect increased MFO-activity at higher CB concentrations or general physiological differences between species.

Using multidimensional gas chromatography (MDGC, Duinker, Schulz & Petrick, 1988a), we found that three toxic CBs, i.e., CB-77 (3,3′,4,4′), CB-118 (2,3′,4,4′,5) and CB-105 (2,3,3′,4,4′) were present in measurable concentrations. CB-77 appeared to contribute about 10% to the peak 110/77 and CB-105 represented about 7% of CB-153 (data for CB-118 are included in the paper). Extremely low levels of CBs can be quantitated by MDGC-ECD. However, the toxic congeners -37, -81, -123, -114, -126, -167, -156, -157, -169 and -189 were below detection limits.

This finding is likely to be of toxicological importance, since the parent compounds of the planar non-ortho chlorine containing congeners of their mono- and di-ortho analogues have the same toxicological mechanisms as 2,3,7,8 TCDD in rats, i.e., they bind to the cytosolic arylhydrocarbon receptor, show a 3-MC or mixed 3-MC and PB-type induction of MFO enzymes, cause liver hypertrophy and hepatic porphyria (Safe, 1984; Safe *et al.* 1985). However, a metabolite of CB-77 showed an entirely different kind of toxicity in mice and rats (Brouwer *et al.* 1986): it caused lowered plasma levels of retinol (vitamin A) and thyroxine.

Studies of persistent CBs in mammal tissues (as has been the case almost exclusively) do not reveal the presence or absence (because of metabolism) of congeners which have structural properties allowing them to be metabolized and possibly to cause harmful effects. Clearly, experimental studies (e.g., such as carried out by Reijnders (1986), involving both metabolizable and persistent congeners are necessary to obtain more insight into their distribution,

Table 7. Hexane extractable lipid based concentration ratios blubber/brain, blubber/heart, blubber/kidney, blubber/liver and blubber/muscle for each CB identified by numbers (Table 2). Presented are the average ratios (avg) and their relative standard deviations (% of average, std%) for the tissues in harbour porpoises (group A) and in other species (group B, Table 1). Concentrations are in nmol g^{-1} lipid. No entry: data do not allow a reliable quantitation

	Blubber/Brain				Blubber/Heart				Blubber/Kidney			
Animal group	A		B		A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
52	4.6	22	5.8	41	1.1	18	0.8	73	1.4	13	1.6	18
49	4.2	14	3.4	50	1.2	16			1.5	25	0.8	100
44	4.1	22	4.1	55	1.4	18	1.0	0	1.3	23	1.4	29
101	4.2	33	8.2	73	1.1	19	0.8	72	1.2	25	1.3	15
149	4.3	60	5.7	49	1.1	50	0.8	76	1.1	61	1.4	8
118	4.6	53	6.0	50	1.4	27	0.8	72	1.3	61	1.4	16
153	3.8	50	5.9	57	0.8	26	0.8	75	0.9	27	1.6	25
138	4.4	50	7.1	46	0.8	23	0.9	76	0.9	25	1.6	21
187	4.0	61	6.6	51	0.6	27	0.9	81	0.7	41	1.6	11
183	4.2	63	6.6	46	0.6	31	0.9	77	0.7	50	1.6	17
177	4.2	52	6.3	51	0.7	27	0.9	80	0.8	42	1.6	19
172	2.9	70	4.1	85	1.6	91	1.7	0	1.4	138	0.8	100
180	4.9	75	6.9	65	2.1	132	0.9	77	0.7	45	1.4	13
201	3.4	69	8.0	82	0.5	49	0.8	81	0.5	76	1.5	18
194	3.5	65	5.0	45	0.5	52	0.7	77	0.6	72	1.5	6
206	4.7	53	3.9	173	0.5	63			0.4	109	0.0	
209	1.7	112	1.2	100	0.8	0			0.3	145	0.0	

	Blubber/Liver				Blubber/Muscle			
Animal group	A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%
52	1.2	26	1.2	11	1.0	41	1.1	21
49	1.2	27	1.1	7	1.0	35	1.2	26
44	1.1	29	1.1	5	0.7	32	0.9	42
101	1.0	25	1.1	6	0.9	26	1.0	26
149	0.8	27	1.1	15	0.8	44	1.0	27
118	0.9	34	1.0	5	0.8	29	1.1	35
153	0.7	26	1.1	13	0.7	44	0.8	37
138	0.8	25	1.1	19	0.7	46	1.0	30
187	0.6	28	1.1	23	0.6	62	0.9	30
183	0.6	32	1.2	22	0.6	68	0.9	29
177	0.6	28	1.1	24	0.7	66	0.9	32
172	0.7	40	0.5	141	0.6	51	0.8	66
180	1.2	146	1.0	35	0.7	62	0.8	36
201	0.4	55	1.0	25	0.5	74	0.8	35
194	0.5	50	1.0	21	0.6	71	0.7	26
206	0.3	108	0.0		0.6	128	0.3	91
209	0.2	150						

kinetics and effects in marine mammals. Identification of metabolites of CBs is complicated, but it can be achieved (Jensen and Jansson, 1976).

Biomagnification of chlorobiphenyls

Figure 11 (left) represents average lipid based concentrations of all CBs listed in Table 2 for Wadden Sea fish, blood of seals fed with Wadden Sea fish (i.e., the Wadden Sea group) for two years, and of porpoise blubber. Concentrations of persistent congeners (group II) in blood of seals of the Wadden Sea group and blubber of porpoises (group A animals) are larger than those in Wadden Sea fish (roughly a factor of 2–4) but those of most of

Table 8. Concentration ratios blubber/brain, blubber/heart, blubber/kidney, blubber/liver and blubber/muscle for each of the pesticides. Presented are average ratios (avg) and their relative standard deviations (% of average, std%) for the tissues in the porpoises (group A) and the other species (group B). Concentrations are in nmol g^{-1} hexane extractable lipid. For numbering of animals, see Table 1

Animal group	Blubber/Brain				Blubber/Heart				Blubber/Kidney			
	A		B		A		B		A		B	
	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
gamma-HCH	6.0	25	4.0	0	1.4	18	1.2	14	2.8	143	1.4	6
alpha-HCH	0.2	34	0.2	15	1.6	16	1.1	6	2.9	85	1.6	8
HCB	2.9	24	3.3	36	0.8	14	0.9	27	0.9	18	1.2	2
Dieldrin	6.6	34	7.8	45	1.2	12	1.0	9	1.4	85	1.5	1
DDD	11.9	35	12.4	46	0.9	40	0.5	40	1.6	83	1.6	10
DDT	16.0	50	23.5	50	7.8	23	27.6	86	19.8	57	31.2	3
DDE	7.7	51	19.4	132	1.0	16	1.2	23	1.4	20	3.0	33

Animal group	Blubber Liver				Blubber/Muscle			
	A		B		A		B	
	avg	std%	avg	std%	avg	std%	avg	std%
gamma-HCH	1.5	43	1.8	14	1.2	64	1.1	13
alpha-HCH	1.7	59	1.4	6	1.7	65	1.1	12
HCB	0.6	40	0.8	1	0.8	59	0.7	4
Dieldrin	1.4	47	1.5	3	3.8	210	1.1	19
DDD	0.9	48	0.9	1	0.9	83	0.6	29
DDT	18.5	52	60.3	11	7.5	61	20.4	74
DDE	1.1	31	1.4	13	1.0	60	1.2	30

the metabolizable congeners (group I) are similar to or smaller than those in fish. Figure 11 (right) shows similar data for Atlantic fish, blood of seals fed with Atlantic fish (i.e., the Atlantic group) for two years and blubber of group B animals. Concentrations in blood of seals of the Atlantic group and blubber of group B animals are one to two orders of magnitude higher than those in Atlantic fish.

Thus a stronger biomagnification is observed in the seals fed with Atlantic mackerel and in the open sea cetaceans than in the seals fed with Wadden Sea fish and the harbour porpoises. However, this conclusion is based on only three chromatograms of Atlantic fish and should be further investigated. Also, the seals fed with Atlantic fish showed decreasing concentrations in blood and may not have reached their equilibrium level with the relatively daily intake of contaminants from oceanic fish. The influence of different rates of metabolism on the degree of biomagnification of specific CB congeners in the different mammals is clearly observed.

Distribution of CBs between tissues

The results discussed above indicate a clear proportionality between the concentration of each CB between different compartments.

Figures 3 and 8 and Table 9 show that the composition of the CB mixtures in blubber, brain, heart, kidney, liver and muscle of each animal investigated are virtually constant. Also, differences between animals of the same species from the same area are only minor, irrespective of absolute concentrations. We have found no indication that the patterns in brain are different from those in other compartments. Tanabe *et al.* (1988), however, reported a larger contribution of early eluting congeners in brains of striped dolphins (it should be noted that these congeners were not specified, but their degree of chlorination can be derived from the Figures).

The present results show that OCs do not accumulate in blubber at the expense of levels in other tissues. Such behaviour can be described by kinetic models, e.g., the typical mammillary model applied in pharmacology and toxicology (Robinson & Roberts 1968; Robinson, Baldwin & Walker, 1969; Moriarty, 1975, 1978). Here, blood plays an important role of the central transport compartment acting as a bridge between the outside world and a series of

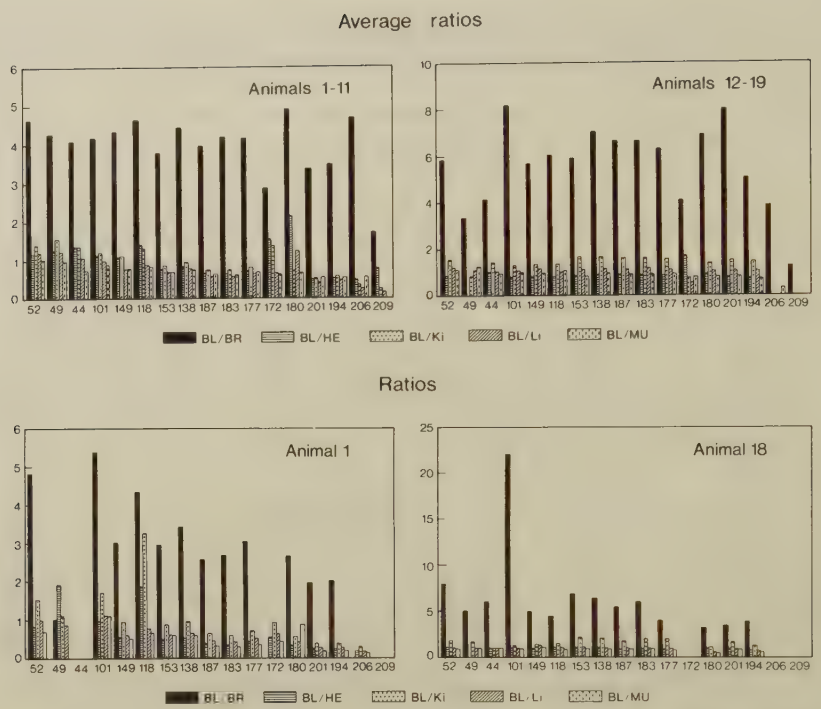

Figure 6. Ratios of concentrations (nmol g^{-1} hexane extractable lipid) of CB congeners (identified by numbers, Table 2) between blubber and other tissues (blubber/brain (BL/BR), blubber/heart (BL/HE), blubber/kidney (BL/KI), blubber/liver (BL/LI), and blubber/muscle (BL/MU)) in animals 1–11 (group A, left) and animals 12–19 (group B, right). Top: average values for groups A and B. Bottom: values for individual animals 1 and 18. The y-axes have different scales.

peripheral compartments (organs) which are more or less independent from each other while maintaining a dynamic balance with the blood and among them (Matthews & Dedrick, 1984; Aguilar, 1985).

A retention kinetics model such as described for the preferential accumulation of Pb in bone tissue (Morgan & Roan, 1972) does not correctly describe the kinetics of organochlorines in fatty tissues of mammals.

Uptake of CBs occurs mainly through food, followed by partitioning into blood cells, haemolymph and blood plasma lipo-proteins and subsequently into the non-polar moieties of different tissues (Matthews & Dedrick, 1984; Harding & Addison, 1986). There are several possibilities for deviations in the correlations between levels in blubber and other compartments (Aguilar, 1985). According to Moriarty (1983) such relations often break down in field populations, essentially because the condition of (near-) steady state is not fulfilled, but our data support this model to a large extent.

By far the most studies of organochlorines in marine mammal tissues are related to their concentrations in blubber. Other tissues have been studied in relatively few cases (Reuthergaard & Knap, 1987). Little is known about the uptake of organochlorines by marine mammals; even less is known about the mechanisms of transport and distribution within an animal. Detailed data involving several tissues are required for developing kinetic transport models in marine mammals. For instance, there is no information for marine mammals on what happens with organochlorines when depot lipids are mobilized for energy purposes. As blubber is a complex and inhomogeneous tissue of variable volume, various problems arise in the interpretation of blubber data only (Aguilar 1985).

We shall analyze here to what extent data on individual congeners in different tissues of various individuals and species may help to understand the distribution mechanisms of lipophilic compounds between tissues.

In order to detect differences in absolute concentrations of each congener between the organs within each of the groups A and B, a one-way analysis of variance was carried out ($\alpha = 0.05$). The results are

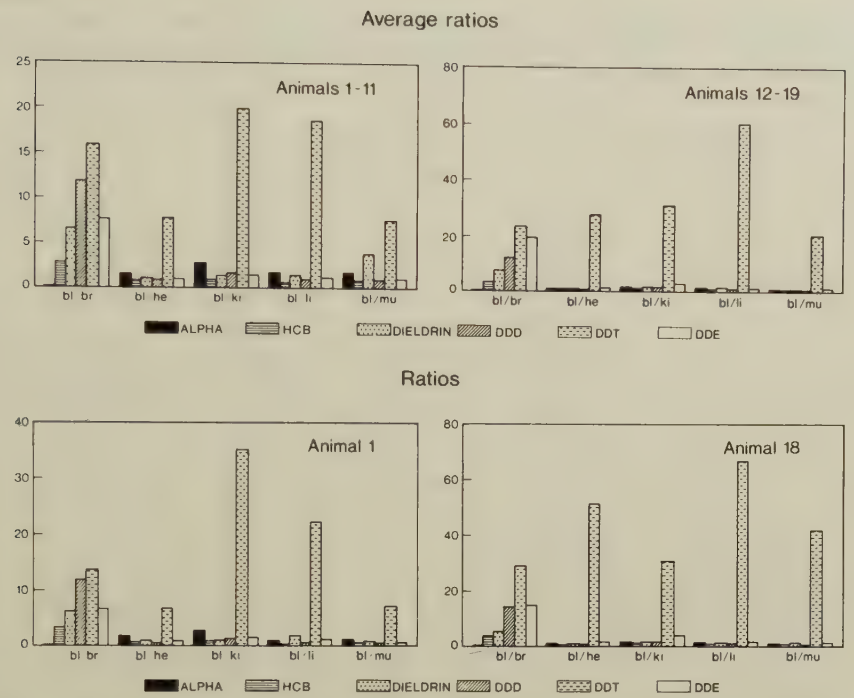

Figure 7. Ratios of concentrations (nmol g^{-1} hexane extractable lipid) of α-HCH (ALPHA), HCB, dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p,p′-DDE (DDE) between blubber and the other tissues (blubber/brain (BL/BR), blubber/heart (BL/HE), blubber/kidney (BL/KI), blubber/liver (BL/LI) and blubber/muscle (BL/MU)) in animals 1–11 (group A, left) and animals 12–19 (group B, right). The y-axes have different scales. Top: average values for groups A and B. Bottom: values for individual animals 1 and 18.

summarized in Table 11. Significant differences are identified in the columns 'ONE WAY' for both groups of animals. Since this analysis identifies whether there is a difference or not, without identifying the cause of any difference, a Tukey test was also performed: the columns 'Tukey test' identify the organs, where the average concentrations differed significantly from others for each CB congener. There are no differences for twelve congeners for both groups, and significant differences for five congeners, which are not the same in groups A and B (except CB Nos. 49 and 44). In all cases where significant differences occur, brain tissue is involved (with the lowest concentrations) and in practically all cases also muscle (with the highest concentrations).

The patterns of persistent CBs (i.e., group II) appear to be very similar in seals and porpoises from the coastal North Sea region. The same similarity applies to the combined 'open sea species' of group B in the present investigation (Fig. 3, Table 9). Also, CB mixtures of persistent congeners analyzed in the fatty protuberance of the melon from Commerson's dolphins caught in the coastal waters of the Kerguelen Islands (Southern Indian Ocean) appear to be very similar (Abarnou, Robineau & Michel, 1986) to the ones that we are reporting here for the North Atlantic. It should be noted that concentration levels in mammal tissues and fish from these remote areas are one to two orders of magnitude below levels reported here for the North Atlantic. Thus it seems that these marine mammal tissues from different parts of the ocean have a more or less constant pattern of relatively persistent CBs. This would suggest that the CB patterns of persistent CBs in the different food sources are also very similar. Their concentrations in mammalian tissues appear to depend on the levels in their food sources and factors like sex and age.

Pesticides

In order to detect significant differences in absolute concentrations of individual pesticides between tissues within each of the groups of animals, a one-way analysis of variance was carried out ($\alpha = 0.05$). The results are given in Table 11. The average

Table 9. Averages and relative standard deviations (% of average, std%) of mol per cent contributions of individual CBs to their sum in each of the tissues of animals 1 11 (group A) and 12 19 (group B). For numbering of animals see Table 1 and of CBs Table 2. No entry: data do not allow a reliable quantitation. Significance levels for differences between groups A and B (two-tailed *t*-test): *$p<0.05$; **$p<0.01$; ***$p<0.001$. Group I and group II congeners are separated by an empty row

	Blubber				Brain				Heart			
Animal group	A		B		A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
52	9.0	25	8.1	13	7.2	30	8.4	18	6.3	17	6.5	8
49	1.0	70	1.2	89	0.5	122	1.0	130	0.8	64	0.6	141
44	0.4	96	1.3	89	0.2	166	1.0	131	0.3	81	1.6	72
101	3.7	58	6.9	28	3.5	53	6.0	33	3.0	62	7.3	19
149	7.1	57	8.9	11	7.4	47	9.8	15	4.3	80	9.6	6
118	2.9	62	5.9	32	3.0	53	6.4	41	1.7	97	6.0	17
153	25.4	9	20.3	27	27.5	12	22.4	14	27.8	3	23.3	3
138	23.0	7	21.0	15	21.3	10	18.7	13	23.5	4	18.9	4
187	8.1	10	7.4	14	9.0	12	7.1	9	10.7	5	8.3	5
183	3.6	12	4.1	13	3.9	17	3.9	13	4.4	7	4.1	8
177	3.6	23	2.5	30	3.7	19	2.6	31	4.7	14	3.1	7
172	0.5	40	0.6	61	0.3	110	0.9	57	0.3	39	0.6	72
180	9.1	31	8.2	40	9.4	43	8.2	33	8.0	63	5.9	8
201	1.4	28	2.3	42	1.9	36	2.1	35	2.4	27	2.7	14
194	0.7	47	1.1	12	0.9	41	0.9	63	1.0	12	1.4	7
206	0.3	111	0.3	134	0.2	190	0.5	119	0.7	71	0.0	
209	0.1	204	0.1	165	0.1	157	0.2	214	0.2	173	0.0	

	Kidney				Liver				Muscle			
Animal group	A		B		A		B		A		B	
CBs	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%	avg	std%
52	5.8	27	6.8	1	5.5	29	6.5	3	5.8	27	6.7	14
49	0.6	71	0.9	103	0.6	70	1.4	71	0.7	57	0.9	95
44	0.2	110	2.4	77	0.3	112	1.8	71	0.3	96	1.1	73
101	2.8	59	8.6	27	2.7	61	7.3	20	2.9	54	6.1	24
149	6.2	64	10.1	1	7.0	57	8.9	13	5.8	63	8.3	13
118	2.3	69	6.7	22	2.7	82	6.4	11	2.3	63	5.2	32
153	25.5	11	21.4	15	25.6	10	22.1	6	25.6	10	22.6	9
138	21.0	9	17.8	14	21.2	9	18.5	5	21.7	10	19.6	9
187	9.8	12	7.6	11	10.0	10	8.0	9	10.1	18	7.6	12
183	4.5	20	3.9	11	4.5	15	4.0	7	4.7	21	4.1	10
177	4.2	16	2.9	18	4.2	14	3.0	10	4.2	32	2.6	32
172	0.5	56	0.6	73	0.5	44	1.0	15	0.6	36	0.8	21
180	11.5	42	6.2	20	10.4	53	6.7	28	10.7	38	9.3	33
201	2.7	37	2.3	9	2.7	34	2.5	17	2.4	38	2.6	31
194	1.2	43	1.2	18	1.2	43	1.3	14	1.3	40	1.3	11
206	0.7	45	0.2	141	0.7	49	0.5	105	0.7	53	1.0	53
209	0.3	80	0.1	141	0.3	64	0.1	141	0.3	70	0.2	106

concentrations of most pesticides in group A are not significantly different between organs of animals of group A, with the exception of α-HCH in brain, HCB in brain and liver, DDD in brain and liver and DDT in blubber compared to all other tissues. In group B animals, differences in average concentrations occur

Table 9. Continued

CBs	Ratio of averages A/B					
	Blubber	Brain	Heart	Kidney	Liver	Muscle
52	1.1	0.9	1.0	0.8	0.8	0.9
49	0.9	0.5	1.5	0.7	0.5	0.8
44	0.3	0.2	0.2*	0.1*	0.1*	0.2*
101	0.5**	0.6**	0.4***	0.3***	0.4***	0.5***
149	0.8	0.8	0.5***	0.6**	0.8	0.7
118	0.5*	0.5**	0.3***	0.4***	0.4***	0.5**
153	1.2*	1.2**	1.2***	1.2**	1.2**	1.1*
138	1.1	1.1*	1.2***	1.2**	1.1*	1.1*
187	1.1	1.3***	1.3***	1.3***	1.3***	1.3**
183	0.9*	1.0	1.1	1.2	1.1*	1.2
177	1.4**	1.4**	1.5***	1.5***	1.4***	1.6**
172	0.8	0.3**	0.5	0.7	0.5	0.7*
180	1.1	1.1	1.4	1.9**	1.5	1.2
201	0.6*	0.9	0.9	1.2	1.1	0.9
194	0.7*	1.0	0.7**	0.9	0.9	1.0
206	1.0	0.4		4.6	1.4	0.7
209	1.6	0.6		2.2	3.1	2.0

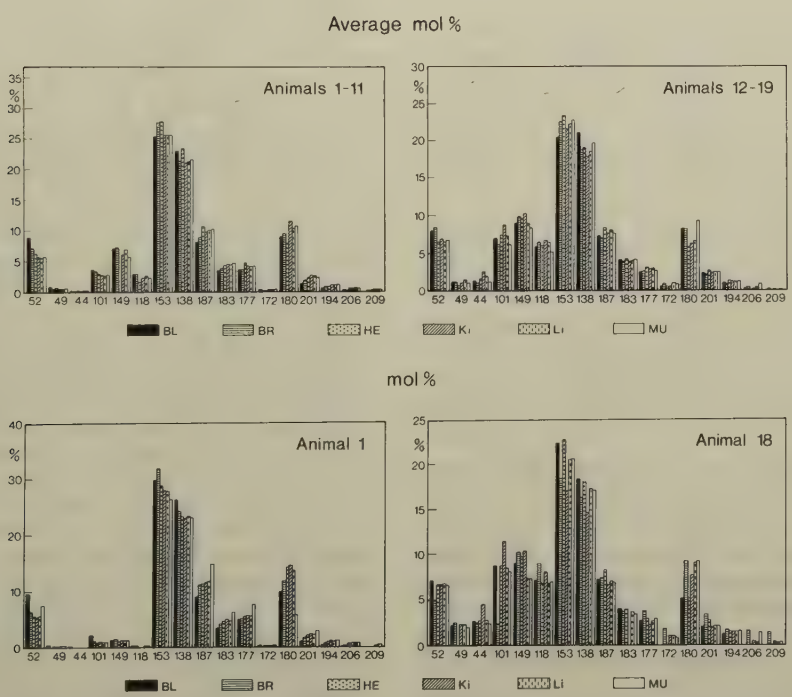

Figure 8. Mol % contributions (based on concentrations in nmol g^{-1} hexane extractable lipid) of CB congeners (identified by numbers, Table 2) to their mixtures in blubber (BL), brain (BR), heart (HE), kidney (KI), liver (LI) and muscle (MU) in animals 1–11 (group A, left) and animals 12–19 (group B, right). Y-axes have different scales. Top: average values of groups A and B. Bottom: values for individual animals 1 and 18.

Table 10. Results of a two-factor analysis of variance (ANOVA) involving the potential systematic influences of the differences between the two groups of animals (A and B) and the differences between the organs, on the relative concentrations of the individual pesticides and CBs. Concentrations were expressed as ratios to DDE and CB No. 153 (X/DDE and X/153, resp.). The underlying significance level was set at $\alpha = 0.05$. The F tests show whether the null-hypothesis of equality of means can be rejected. This is the case when the p values of the marginal F distributions are smaller than $\alpha = 0.05$. In those cases, significant differences are present. Given are the p values for the main (overall) effect and the tests of the null-hypothesis of equality in the two groups and in the organs. The ratios are statistically not significantly different for $p > \alpha$

Compounds	p values		
	Groups of animals	Organs	Overall effect
Pesticides			
γ-HCH	0.00	0.97	0.00
α-HCH	0.01	0.00	0.00
HCB	0.00	0.00	0.00
Dieldrin	0.00	0.74	0.03
DDD	0.00	0.28	0.00
DDT	0.00	0.00	0.00
Chlorobiphenyls			
52	0.01	0.01	0.00
49	0.01	0.73	0.16
44	0.00	0.70	0.00
101	0.00	0.40	0.00
149	0.00	0.49	0.00
118	0.00	0.60	0.00
138	0.18	0.19	0.15
187	0.85	0.59	0.69
183	0.05	0.70	0.39
177	0.00	0.43	0.02
172	0.00	0.76	0.00
180	0.94	0.74	0.84
201	0.00	0.12	0.12
194	0.00	0.32	0.06
206	0.47	0.03	0.04
209	0.47	0.38	0.40

for α-HCH and γ-HCH in brain, for dieldrin and DDD between brain and muscle, for DDT in blubber and for DDE compared to blubber and muscle.

We shall use concentration data measured in blubber, brain, heart, kidney, liver and muscle of harbour porpoises and species of the open sea, in blood of seals fed with Atlantic fish (Atlantic group), Atlantic fish (mackerel) and in Wadden Sea fish. The latter were sampled at 17 stations in the Dutch and German Wadden Sea during two different periods in 1979. Whole fish (plaice and flounder) and homogenates of shrimps were used for analysis. Contributions in shrimp and Wadden Sea fish are very similar. The composition of the pesticides mixture differed between the Wadden Sea and Atlantic fish (Fig. 12). The highest contributions in the Wadden Sea fish are those of HCB and DDD (40 and 30 mol % in the mixtures, reflecting the relative concentrations of these pesticides in Dutch coastal waters (Duinker & Hillebrand, 1979a). DDE and DDT were the dominant contributors in the Atlantic fish (45 and 20 mol %, respectively, Fig. 12).

Comparison of the compositions of the pesticide mixtures (Fig. 12) and the individual concentrations in fish, blubber and liver in the coastal samples (Fig. 13) show that γ-HCH, α-HCH and HCB are not biomagnified in liver and blubber. Figs. 12 and 13 summarize also the corresponding data for the Atlantic group of mammals. γ-HCH, α-HCH, HCB, dieldrin, DDD and DDT occur at lower relative concentrations, while DDE is the dominant contributor to the mixtures (up to 85% in liver, Fig. 12). This high contribution is larger than in most other data reported (see Aguilar, 1985). Like in the coastal species, γ-HCH, α-HCH and HCB are not biomagnified in blubber and liver. DDD and DDE are biomagnified in blubber and liver and DDT also in blubber but not in liver.

In the literature, the contributions of p,p'-DDT, p,p'-DDD and p,p'-DDE have often been added and described as $\sum$-DDTs. Figure 12 shows that the relative contributions of each compound to the mixture may vary considerably between sample types. The increase in the mol % contribution of DDE to $\sum$-DDT in open sea cetaceans in the series food < blubber < liver (up to 85%) parallels the porpoise data, but the relative contributions of DDE in the porpoise data are considerably smaller for all sample types (Fig. 12). The mol % contributions of DDD are larger in each of the corresponding coastal or Wadden Sea animals. A different situation occurs for DDT, where mol % contributions are much lower in Wadden Sea fish than in Atlantic fish, although relative contributions to blubber and liver of cetaceans are larger. Comparison of average concentrations in blubber and liver of the two groups of animals A and B show that the ratio:–
{blubber A/liver A}/{blubber B/liver B} is very similar for DDD, DDT and DDE, suggesting that similar mechanisms may be operative in the animal groups A and B.

The relatively high concentration of α-HCH in brain tissue compared to other compartments in especially porpoises has also been reported for Wadden Sea seals (Duinker & Hillebrand, 1979b). For all other—less polar—compounds, brain levels are the lowest. An hematoencephalic barrier was assumed to be responsible for this phenomenon (Franck, Ronald & Braun, 1973), but lipid com-

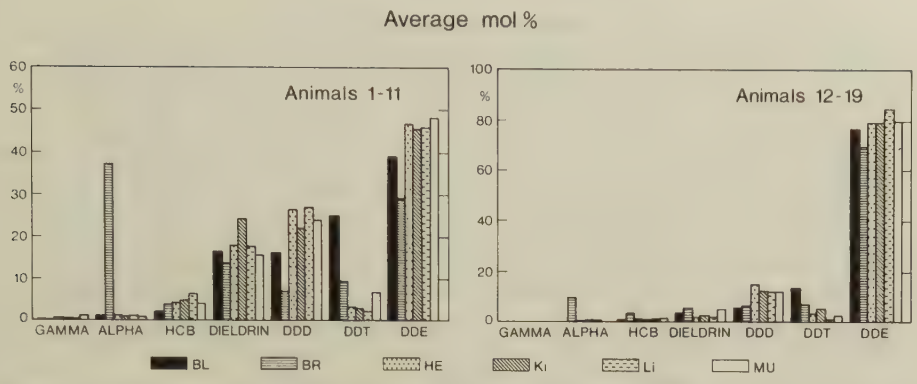

Figure 9. Average mol % contributions (based on concentrations in nmol g^{-1} hexane extractable lipid) of γ-HCH (GAMMA), α-HCH (ALPHA), HCB, dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p.p′-DDE (DDE) to their mixtures in blubber (BL), brain (BR), heart (HE), kidney (KI), liver (LI) and muscle (MU) in animals 1 11 (group A, left) and animals 12–19 (group B, right). Y-axes have different scales.

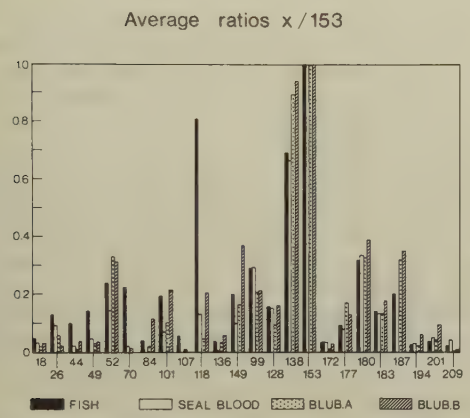

Figure 10. Average concentration ratios (X/153) for CB congener X (identified by numbers, Table 2) relative to CB-153 in Wadden Sea fish, seal blood, blubber (BLUB) of North Sea porpoises of group A (BLUB.A) and blubber of the species of group B (BLUB.B). The order of CBs on the x-axis is different from that in the preceding graphs. They have been arranged in the order of increasing CB number for both group I congeners (CB-18 through CB-149) and group II congeners (CB-153 through CB-209).

position was considered to be a more plausible explanation by others (Roberts, de Freitas & Gidney, 1977; Mitchell, Plack & Thomspon, 1977). The large fraction of relatively polar phospholipids in brain would account for the larger concentrations of relatively polar compounds such as α-HCH (Kawai *et al.*, 1988).

Since e.g., CBs show the same patterns in all tissues, including brain, the present results would support the latter hypothesis. The distribution patterns of pesticides in different tissues and organs of animals from the two groups are considerably more variable than those of the CBs. This reflects the different chemical nature of the pesticides.

In order to test any difference in affinity of the pesticides for specific tissue lipids, a one-way analysis of variance was carried out, involving concentration ratios blubber/brain, blubber/heart, etc., within each of the groups A and B. The underlying significance level was set at $\alpha = 0.05$. The results are given in Table 12. Significant differences occur in the blubber/brain ratios for γ-HCH, HCB, DDD and DDE relative to all other ratios and for α-HCH relative to blubber/kidney in group A. These differences are reflected in the average values as shown in Figure 7. Average blubber/brain ratios in group B are different from all other ratios for γ-HCH, dieldrin and DDD, more complicated relations occur for α-HCH and HCB. For DDE, differences between tissues are not significant, reflecting the large standard deviations (Table 8).

Without more fundamental studies on the kinetics of pesticides in mammals being available, no more quantitative relations can be established from the present data.

Lipid compositions

The concentrations of congeners on a wet weight basis vary considerably between tissues of one animal. The largest part of these differences disappear when concentrations are expressed on an hexane-extractable lipid basis (Table 4, Fig. 4), but smaller differences remain. Especially levels in brain appear to be considerably lower, except for α-HCH, which showed considerably higher lipid based concentrations in brain compared to all other tissues. This was also reported by Kawai *et al.* (1988). These authors also investigated the types of lipid classes present in different cetacean tissues. In brain the total lipid fraction mainly consisted of phospholipids

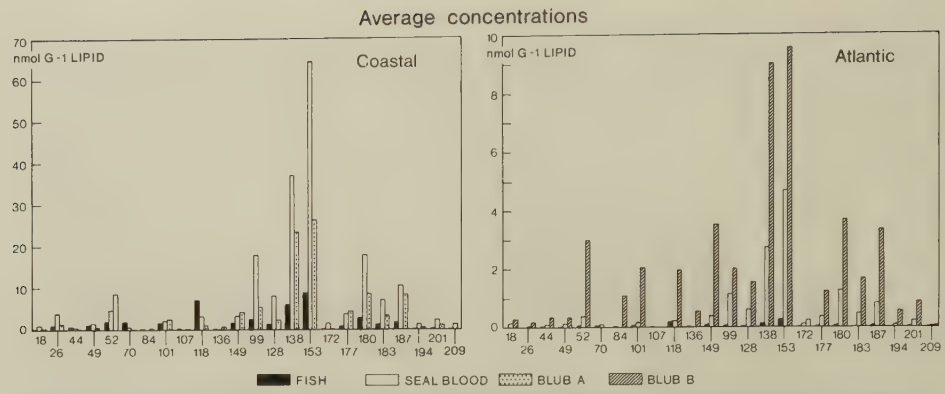

Figure 11. Average concentrations (nmol g ^{1} hexane extractable lipid) of CBs (identified by numbers, Table 2) in left: Wadden Sea plaice (FISH), blood of seals fed with Wadden Sea fish (SEAL BLOOD) and blubber of North Sea porpoises (BLUB. A); right: Atlantic fish (mackerel, FISH), blood of seals fed with Atlantic fish (SEAL BLOOD) and blubber of other species (group B animals, BLUB. B). The y-axes have different scales.

(50–70%) and total cholesterol (15–30%). Triacylglycerols constituted only a minimal fraction in brain and increased in the series < liver, heart < muscle < kidney < blubber, where triacylglycerols were the only lipid class present. In general, the triacylglycerol fraction increased with increasing amounts of total extractable lipids of the different organs, but brain formed an exception to this rule in that it was relatively lipid rich, but the contribution of triacylglycerols was minimal. In conclusion, organochlorines appear to follow the basic chemical rule that a given compound dissolves best in a 'solvent' of similar polarity.

The method involving the use of an organic solvent (or a mixture of solvents) to estimate the amount of 'extractable' lipids is an admittedly inadequate way to represent the 'site' of CBs in animal tissues, taking into account the complex nature of lipids and the different specific affinities of individual compounds for different lipid constituents and other apolar moieties (Kawai and Fukushima 1981). For instance, Tanabe *et al.* (1988) argued that the content of the sum of triacylglycerols and free fatty acids in a tissue would be a better basis for expressing the concentration of lipophilic compounds in that tissue. The large contribution of waxes to blubber, muscle and organs of the sperm whale may partly account for the low extractable lipid-based concentrations of all CBs in all tissues of animal 15, but other factors like feeding habits may also contribute.

The suggestion of Tanabe *et al.* (1988) is favourable to the standardisation of concentration levels on triacylglycerols alone, since blubber did not show higher OC concentrations than less total-extractable lipid- and triacylglycerol-rich tissues such as liver, muscle, kidney and heart in the present study. This observation suggests that the covariance of lipids and chlorinated biphenyls is a function of specific affinity of CBs for certain types of lipids and not a non-specific affinity for all lipids and other hydrophobic moieties (Phillips, 1986).

We feel therefore that considerably more work is necessary to develop methods for more precise and accurate correlations with the actual apolar terminals in tissues which secure the lipophilic organochlorines being studied. In particular, it seems highly desirable to adopt a standardized procedure for analyzing a broad spectrum of lipid classes as has been performed in some cases (Roberts, de Freitas & Gidney, 1977; Mitchell, Plack & Thomspon, 1977; Tanabe *et al.*, 1981a,b; Schneider, 1980, 1982; Kawai *et al.*, 1988).

It was mentioned above that the mol % contributions of several pesticides differ considerably between tissues. These observations would result if lipids of the various tissues have larger affinity differences for pesticides than for CBs. The lipid composition of a given tissue type is expected to vary only slightly between different animals (Tanabe *et al.*, 1988). Therefore, each block of bars in Figure 2 (grouped per compound in each animal) would be expected to be qualitatively similar for each compound in all animals. The overall height of the block in this figure would vary between animals according to varying burdens of the compounds. Also, we would expect quite large differences between blocks (representing different pesticides). This expectations are confirmed to a large extent by the data (Figs. 2 and 9).

Comparison between animals found dead and those kept in captivity

Several authors have expressed serious concern whether the levels of OCs determined in organisms found dead can be considered as representative for

Table 11. Results of a ONE-WAY analysis of variance of the absolute (lipid based) concentrations of the pesticides and chlorobiphenyls in each of the two groups of animals (A and B), represented by the p values ($\alpha = 0.05$) in the columns 'ONE WAY'. The ratios are not significantly different for $p > \alpha$. Also a Tukey test (a modified *t*-test) was carried out. The significant differences of the average concentration of any compound between tissues are identified in the columns 'Tukey test'. If one tissue is given, its average concentration was significantly different from those of all other tissues (bl = blubber; br = brain; he = heart; ki = kidney; li = liver; mu = muscle). Expressions like 'br vs bl,li,mu' indicate that the average brain concentration was significantly different from those of blubber, liver and muscle (implying that brain, heart and kidney formed an homogeneous group, and similarly blubber, heart, kidney, liver and muscle formed another homogeneous group)

	Group A animals		Group B animals	
Compounds	ONE WAY p	Tukey test	ONE WAY p	Tukey test
Pesticides				
γ-HCH	0.08	—	0.00	br
α-HCH	0.00	br	0.00	br
HCB	0.06	br vs li	0.03	—
Dieldrin	0.27	—	0.01	br vs mu
DDD	0.08	br vs li	0.00	br vs mu
DDT	0.00	bl	0.00	bl
DDE	0.39	—	0.00	br vs bl,mu
CBs				
52	0.04	br vs mu	0.03	—
49	0.00	br vs bl,li,mu	0.02	bl vs br
44	0.04	br vs mu	0.01	br vs bl,mu
101	0.00	br vs bl,li,mu	0.02	—
149	0.31	—	0.03	—
118	0.29	—	0.02	bl vs br
153	0.06	—	0.06	—
138	0.06	br vs mu	0.03	—
187	0.08	—	0.03	br vs mu
183	0.15	—	0.03	—
177	0.06	—	0.07	—
172	0.11	—	0.03	—
180	0.37	—	0.07	—
201	0.17	—	0.15	—
194	0.27	—	0.02	br vs mu
206	0.03	—	0.03	—
209	0.14	—	0.18	—

levels in the population (Aguilar, 1985; Olsson, 1986). Bergman, Olsson & Reuthergaardh (1981) found that average PCB concentrations in animals found dead were two times higher than those in actively killed ones. Stranded organisms may have suffered from diseases before death occurred and decay afterwards. The constancy of the composition of mixtures in different tissues of animals, sampled once found dead and of those which died at the Dolfinarium at Harderwijk, as well as the good correlation with seal blood taken from animals in captivity suggest that the problem, if existing, affects all CBs similarly. An important point might be that blood as transport compartment, stops flowing after death.

There are nevertheless several reasons (e.g., tissue inhomogeneities, differences in sample treatment, instrumental analytical and data reporting techniques) (Aguilar, 1985; Reuthergaard & Knap, 1987) why data on organochlorine compounds in marine mammal tissues reported by different authors may not be well comparable (Andersen & Rebsdorff, 1976; Gaskin, Holdrinet & Franck, 1976; Drescher, Harms & Huschenbeth, 1977; Donkin, Mann & Hamilton, 1981; Wagemann & Muir, 1981).

Conclusions

Chlorinated biphenyls

Variable concentrations of individual chlorinated biphenyls (CBs) and pesticides were established in

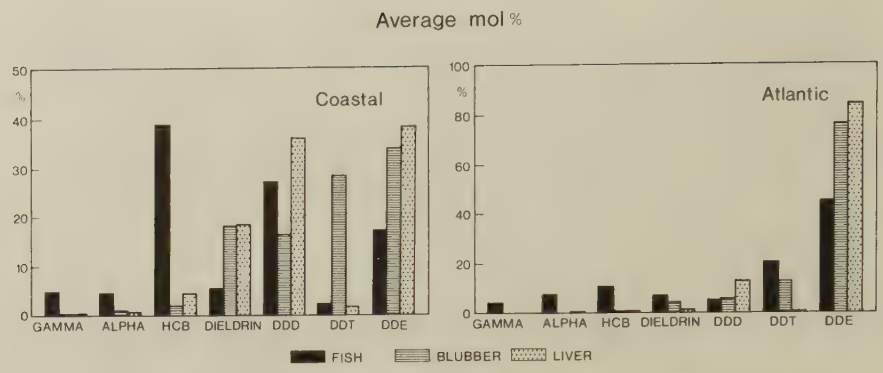

Figure 12. Average mol % contributions of γ-HCH (GAMMA), α-HCH (ALPHA), HCB, dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p,p′-DDE (DDE) to their mixtures in Wadden Sea fish (left) and Atlantic fish (right) and blubber and liver of animals 1–11 (North Sea porpoises, group A, left) and animals 12–19 (other species, group B, right).

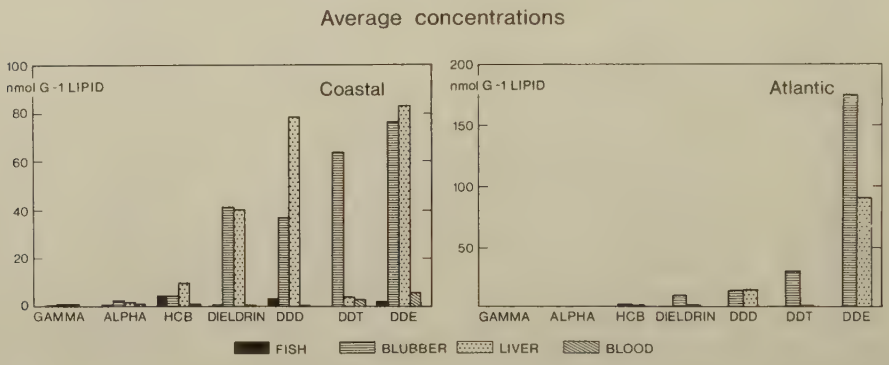

Figure 13. Average concentrations (nmol g ^{1} hexane extractable lipid) of γ-HCH (GAMMA), α-HCH (ALPHA), HCB, dieldrin, p,p′-DDD (DDD), p,p′-DDT (DDT) and p,p′-DDE (DDE) in fish, blubber, liver and blood. Left: Wadden Sea fish, and blubber, liver and blood of North Sea porpoises (group A, animals 1–11); Right: Atlantic fish, and blubber and liver of the other species (group B, animals 12–19).

different tissues of the same organism and between organisms from the coastal area, the open North Sea and the western Atlantic. Differences in lipid based concentration in the same animal were generally lower than between animals.

Maximum concentrations of CBs and pesticides were of the same order of magnitude (50–100 nmol g^{-1} lipid with DDE values up to 500). $\sum$-PCB/$\sum$-DDT ratios which have been used frequently in the literature tend to obscure this observation: a value around 10 has been considered characteristic for several areas. Moreover, sums of concentrations do not reveal and distinguish the compositions of the mixtures. Significant differences in the relative amounts of p,p′-DDT, p,p′-DDD and p,p′-DDE have been established between tissues (large contributions of DDT in blubber and of DDE in liver and considerable differences between the porpoises and the open sea species) which make comparisons of their sums scientifically irrelevant.

The mixtures of persistent CBs appeared to be very similar in different mammal tissues from the Dutch coastal area, the North Sea, the western Atlantic and literature data from the Southern Indian Ocean. The mixtures of CBs in marine mammal tissues and organs are dominated by highly chlorinated biphenyls. This does not reflect the composition in ingested food, as was also suggested by Phillips (1986).

The differences in biomagnification can be interpreted in terms of the number and relative positions of chlorine atoms in the molecular framework, suggesting biotransformation to be the most probable cause for the decreased biomagnification of several congeners. The apparent contribution of biotransformation to the kinetics of PCBs in marine mammals was seals > porpoises > open sea cetaceans. The differences between these classes can be caused by physiological differences or a higher degree of mixed function oxygenase induction at

Table 12. Results of a ONE-WAY analysis of variance (ONE-WAY) of pesticide concentration ratios in the different organs of each of the two groups of animals A and B. bl/br = blubber/brain; bl/he = blubber/heart; bl/ki = blubber/kidney; bl/li = blubber/liver; bl/mu = blubber/muscle. The underlying significance level was set at $\alpha = 0.05$. p values are given in the appropriate columns 'ONE WAY'. Also a Tukey test (a modified *t*-test) was carried out. In the corresponding columns, those ratios are identified that differed significantly from others; e.g., an entry bl/br indicates that the average blubber/brain ratio differed significantly from all other average ratios for that compound. Similarly, an entry 'bl/br vs bl/ki' indicates that the average blubber/brain ratio differed significantly from bl/ki, while average bl/br, bl/he, bl/li, bl/mu values and also the group of average bl/he, bl/ki, bl/li, and bl/mu values formed two homogeneous groups

	Group A animals		Group B animals	
Compounds	ONE WAY p	Tukey test	ONE WAY p	Tukey test
γ-HCH	0.00	bl/br	0.00	bl/br
α-HCH	0.01	bl/br vs bl/ki	0.00	bl/br; and bl/he vs bl/ki and bl/ki vs bl/mu
HCB	0.00	bl/br	0.00	bl/br vs all but bl/ki
Dieldrin	0.05	—	0.00	bl/br
DDD	0.00	bl/br	0.00	bl/br
DDT	0.00	—	0.08	—
DDE	0.00	bl/br	0.36	—

higher levels of PCBs. CB-congeners with vicinal H-atoms only in the m,p position were apparently metabolized in seals, but were persistent in cetaceans. CB-congeners with vicinal H-atoms in the o,m position with one ortho-chlorine at maximum, were metabolized in each case. Although CBs with either vicinal H-atoms only in the m,p position or only in the o,m position with two ortho-chlorines were persistent in cetaceans, congeners possessing both features were apparently metabolized.

Concentrations in brain are lower than in all other organs investigated. Enriched brain concentrations of early eluting congeners (involving metabolizable ones), as found by Tanabe *et al.* (1988) were not found here.

The biomagnification and distribution of CBs is probably due to specific affinities of CBs for lipids rather than to a non-specific affinity of CBs for all lipids (Phillips, 1986). This is supported by the general finding that the lipid based concentrations of CBs decreased in the order brain < heart, kidney, liver < blubber and the decrease in biomagnification with increasing degree of chlorination in blubber of harbour porpoises.

No indication was found for accumulation in blubber at the expense of other tissues since patterns were virtually identical. Comparison with data on experiments with PCBs and seals (Boon *et al.*, 1987) shows that biomagnification of metabolizable congeners was lower in blood, suggesting that the uptake from food into blubber is more effective and/or elimination from blubber is faster than for blood. Net transfer from food to faeces, blood and blubber appeared to be very similar for persistent congeners.

It is highly desirable that analyses of OCs be accompanied by lipid speciation studies, for which methods have been proposed in the literature. This would further increase the usefulness of CBs as model compounds for the behaviour of lipophilic organic compounds in the environment. It is therefore essential that in studies of PCBs and other mixtures of organochlorine compounds, individual compounds are studied, rather than undefined mixtures.

Pesticides

The patterns of pesticides are considerably more variable than those of CBs, reflecting the larger differences in molecular structures and properties. Relatively large differences were found in the compositions of the pesticide mixtures between tissues of the same animal and between those of the porpoises and the other species from the open North Sea and western Atlantic. DDE is the dominant contributor in the tissues of the latter group (up to 85% of the mixture in liver). Also in Atlantic fish (mackerel) DDE was found to be the dominant contributor, in contrast to the situation in Wadden Sea fish where DDD is the dominant contributor, reflecting the relative concentrations of DDT, DDT and DDE in solution in Dutch coastal waters (Duinker & Hillebrand, 1979a).

The concentrations of some OCs pesticides in marine organisms appear to be similar to, or even larger than those of individual chlorobiphenyls. The popular method to express the relative concentrations of PCBs and DDTs as the ratio $\sum$PCB/$\sum$DDT (often found to be around 10) therefore underestimates the possible role of OC pesticides for marine mammals compared to CBs. We recommend that OC pesticides are analysed in marine mammal tissues, in addition to individual CBs.

α-HCH is a relatively polar compound. Its relative concentration is low in all organs, except in brain. This is more likely to result from the high fraction of the polar phospholipids in brain lipids than due to the presence of a hematoencephalic barrier.

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References

Abarnou, A., Robineau, D. & Michel, P. (1986). Contamination par les organochlores des dauphins de Commerson des iles Kerguelen. *Ocean. Acta* **9,** 19–29.

Abdel-Hamid, F. M., Moore, J. A. & Matthews, H. B. (1981). A comparative study of 3,4,3′,4′-tetrachlorobiphenyl in male and female rats and in female monkeys. *J. Toxicol. Environ. Health* **7,** 181–191.

Addison, R. F. & Smith, T. G. (1974). Organochlorine residue levels in arctic ringed seals: Variation with age and sex. *Oikos* **25,** 335–337.

Addison, R. F. & Brodie, P. F. (1977). Organochlorine residues in maternal blubber, milk, and pup blubber from grey seals (*Halichoerus grypus*) from Sable Island, Nova Scotia. *J. Fish. Res. Bd. Can.* **34,** 937–941.

Aguilar, A. (1985). Compartmentation and reliability of sampling procedures in organochlorine pollution surveys of cetaceans. *Res. Rev.* **95,** 91–114.

Almkvist, L. Baltic Marine Mammals—A status report. *ICES CM 1982/N*: 16, 19 pp.

Alzieu, C., Duguy, R. & Babin, P. (1982). Pathologie des Delphinidae: contamination foetale et neo-natale par les PCB. Lesions cutanée ulceratives. *Rec. Trav. Ins. Peches Mar.* **46,** 157–166.

Andersen, S. H. & Rebsdorff, A. (1976). Polychlorinated hydrocarbons and heavy metals in harbour porpoise (*Phocoena phocoena*) and whitebeaked dolphins (*Lagenorhynchus albirostris*) from Danish waters. *Aquat. Mamm.* **4,** 14–20.

Anonymous (1987). ICES/IOC/UNEP Review of contaminants in marine mammals. *ICES CM. 1987/N*: 8.

Ballschmiter, K. & Zell, M. (1980). Analysis of polychlorinated biphenyls (PCB) by glass capillary gas-chromatography. Composition of technical Aroclor and Clophen-PCB mixtures. *Z. Anal. Chem.* **302,** 20–31.

Béland, J., Michaud, R. & Martineau, D. (1987). Recensements de la population de belugas du Saint-Laurent en 1985 par embarcations. *Rap. tech. can. sci. halieut, aquat.* No. 1545.

Bergman, A., Olsson, M. & Reutergaard, L. (1981). Lowered reproduction rate in seal population and PCB concentration. *ICES CM 1981/N*: 10.

Boon, J. P., Reijnders, P. J. H., Dols, J., Wensvoort, P. & Hillebrand, M. T. J. (1987). The kinetics of individual polychlorinated biphenyl congeners in female harbour seals (*Phoca vitulina*), with evidence for structure-related metabolism. *Aquat. Toxicol.* **10,** 307–324.

Brouwer, A., van den Berg, A. J., Blaner, W. S. & Goodman, D. S. (1986). Transthyretin (prealbumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. *Chemosphere* **15,** 1699–1706.

DeLong, R. L., Gilmartin, W. G. & Simpson, J. G. (1973). Premature births in California sea lions: Association with high organochlorine pollutant residue levels. *Science* **181,** 1168–1170.

Donkin, P., Mann, S. V. & Hamilton, E. I. (1981). Polychlorinated biphenyl, DDT and dieldrin residues in grey seal (*Halichoerus grypus*) males, females and mother-foetus pairs sampled at the Farne Islands, England, during the breeding season. *Sci. Total Envir.* **19,** 121–142.

Drescher, H. E., Harms, U. & Huschenbeth, E. (1977). Organochlorines and heavy metals in the harbour seal (*Phoca vitulina*) from the German North Sea coast. *Mar. Biol.* **41,** 99–106.

Duinker, J. C. & Hillebrand, M. T. J. (1979a). Behaviour of PCB, pentachlorobenzene, hexachlorobenzene, α-HCH, γ-HCH, β-HCH, dieldrin, endrin and p,p′-DDD in the Rhine-Meuse estuary and the adjacent coastal area. *Neth. J. Sea Res.* **13,** 256–281.

Duinker, J. C. & Hillebrand, M. T. J. (1979b). Mobilization of organochlorines from female lipid tissue and transplacental transfer to fetus in a harbour porpoise (*Phoceoena phocoena*) in a contaminated area. *Bull. Environ. Contam. Toxicol.* **23,** 728–732.

Duinker, J. C. & Hillebrand, M. T. J. (1983a). Characterization of PCB components in Clophen formulations by capillary GC-MS and GC-ECD techniques. *Env. Sci. Technol.* **17,** 449–456.

Duinker, J. C. & Hillebrand, M. T. J. (1983b). Determination of selected organochlorines in seawater. In: Methods of seawater analysis (eds. K. Grasshoff, M. Ehrhardt & K. Kremling), chapter 12.4, pp. 290–309. Verlag Chemie.

Duinker, J. C., Schulz, D. E. & Petrick, G. (1988a). Multidimensional gas chromatography with electron capture detection for the determination of toxic congeners in polychlorinated biphenyl mixtures. *Anal. Chem.* **60,** 478–482.

Duinker, J. C., Schulz, D. E. & Petrick, G. (1988b). Selection of chlorinated biphenyl congeners for analysis in environmental samples. *Mar. Poll. Bull.* **19,** 19–25.

Duinker, J. C., Knap, A. H., Binkley, K. C., van Dam, G. H., Darrel-Rew, A. & Hillebrand, M. T. J. (1988). Method to represent the qualitative and quantitative characteristics of PCB mixtures. Marine mammal tissues and commercial mixtures as examples. *Mar. Pollut. Bull.* **19,** 74–79.

Fuller, G. B. & Hobson, W. C. (1986). Effects of PCBs on reproduction in mammals. In: PCBs and the environment (ed. J. S. Waid) CRC Press. Vol. II, 101–125.

Frank, R., Ronald, K. & Braun, A. H. (1973). Organochlorine residues in harp seals (*Pagophilus groenlandicus*) caught in eastern Canadian waters. *J. Fish. Res. Bd. Canada* **30,** 1053–1063.

Gage, J. C. & Holm, S. (1976). The influence of molecular structure on retention and excretion of polychlorinated biphenyls by the mouse. *Toxicol. Appl. Pharmacol.* **36,** 555–560.

Gaskin, D. E., Holdrinet, M. & Frank, R. (1976). DDT residues in blubber of harbour porpoise, *Phocoena phocoena*, from eastern Canadian waters during the five year period 1969–1973. ACMRR/MM/SC/96 pp. 1–11.

Gilmartin, W. G., DeLong, R. L., Smith, W., Sweeney, J. C., DeLappe, B. W., Risebrough, R. W., Griner, L. A., Dailey, M. D. & Peakall, D. B. (1976). Premature parturition in the California sea lion. *J. Wildl. Diseases* **12,** 104–115.

Hamelink, J. L., Waybrant, R. C. & Ball, R. C. (1971). A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. *Trans. Am. Fish. Soc.* **100,** 207–214.

Harding, G. C. & Addison, R. F. (1986). Accumulation and effects of PCBs in marine invertebrates and vertebrates. In: PCBs and the environment (ed. J. S. Waid) CRC Press. Vol. II, 9–30.

Helle, E. (1980). Lowered reproductive capacity in female ringed seals (*Pusa hispida*) in the Bothnian Bay, northern Baltic Sea, with special reference to uterine occlusions. *Ann. Zool. Fenn.* **17,** 147–158.

Helle, E. (1981). Reproductive trends and occurrence of organochlorines and heavy metals in the Baltic seal populations. *ICES CM 1981/E*: 37.

Helle, E. (1986). The decrease in the ringed seal population of the Gulf of Bothnia in 1975–1984. *Finnish Game Res.* **44,** 28–32.

Helle, E., Olsson, M. & Jensen, S. (1976a). DDT and PCB levels and reproduction in ringed seals from the Bothnian Bay. *Ambio* **5,** 188–189.

Helle, E., Olsson, M. & Jensen, S. (1976b). PCB levels correlated with pathological changes in seal uteri. *Ambio* **5,** 261–263.

Hook, O. & Johnels, A. G. (1972). The breeding and distribution of the grey seal (*Halichoerus grypus*. Fab) in the Baltic Sea, with observations on other seals of the area. *Proc. R. Soc. Lond. Ser. B.* **182,** 37.

Jensen, S. & Jansson, B. (1976). Anthropogenic substances in seals from the Baltic: Methylsulfone metabolites of polychlorinated biphenyls and DDE. *Ambio* **5,** 257–260.

Kawai, S. & Fukushima, M. (1981). Relation between lipid composition and the concentrations of organochlorine compounds in the various organs of striped dolphins *Stenella coeruleoalba*. In: *Studies on the levels of organochlorine compounds and heavy metals in marine organisms* (ed. T. Fujiyama) Univ. of Ryukyus.

Kawai, S. M., Fukushima, N., Miyazaki, N. & Tatsukawa, R. (1988). Relationships between lipid composition and organochlorine levels in the tissues of striped dolphin. *Mar. Pollut. Bull.* **19,** 129–133.

Martineau, D., Béland, P., Desjardins, C. & Lagacé, A. (1987). Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence estuary, Quebec, Canada. *Arch. Environ. Contam. Toxicol.* **16,** 137–147.

Matthews, H. B. & Dedrick, R. L. (1984). Pharmacokinetics of PCBs. *Ann. Rev. Pharmacol. Toxicol.* **24,** 85–103.

Mitchell, A. I., Plack, P. A. & Thompson, I. M. (1977). Relative concentrations of C14-DDT and of two polychlorinated biphenyls in the lipids of cod tissues after a single oral dose. *Arch. Environ. Contam. Toxicol.* **6,** 525.

Moriarty, F. (1975). Exposure and residues. In: Organochlorine insecticides: Persistent organic pollutants (ed. F. Moriarty) Academic Press, London, p. 29.

Moriarty, F. (1978). Terrestrial animals. In: *Principles of ecotoxicology* (ed. G. C. Butler) Wiley, N. York, p. 169.

Moriarty, F. (1983). Ecotoxicology. The study of pollutants in ecosystems. Academic Press, London.

Morgan, D. P. & Roan, C. C. (1972). Loss of DDT from storage in human body fat. *Nature (London)* **238,** 221.

Muir, D. C. G., Norstrom, R. J. & Simon, M. (1988). Organochlorine contaminants in arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane-related compounds. *Environ. Sci. Technol.* **22,** 1071–1079.

Mullin, M. D., Pochini, C. M., McCrindle, S., Romkes, M., Safe, S. H. & Safe, L. (1984). High resolution PCB analysis: Synthesis and chromatographic properties of all 209 PCB congeners. *Env. Sci. Technol.* **18,** 468–476.

Norstrom, R. J., Simon, M., Muir, D. C. G. & Schweinsburg, R. E. (1988). Organochlorine contaminants in arctic marine food chains: identification, geographical distribution, and temporal trends in polar bears. *Environ. Sci. Technol.* **22,** 1063–1071.

Olsson, M. (1986). PCBs in the Baltic environment. In: *PCBs and the environment* (ed. J. S. Waid) CRC Press. Vol. III, 181–208.

Olsson, M., Johnels, A. G. & Vaz, R. (1975). DDT and PCB levels in seals from Swedish waters. The occurrence of aborted seal pups. *Nat. Swed. Environ. Prot. Board PM* **591,** 43–65.

Phillips, D. J. H. (1986). Use of organisms to quantify PCBs in marine and estuarine environments. In: *PCBs and the environment* (ed. J. S. Waid) CRC Press. Vol. II, 127–181.

Reuthergaard, L. & Knap, A. H. (1987). Overview of organochlorine compound analysis in marine mammals. *ICES Mar. Chem. Work. Group. 1987/7.2.2*, mimeo, 23 pp.

Reijnders, P. J. H. (1978). Recruitment in the harbour seal (*Phoca vitulina*) population in the Dutch Wadden Sea. *Neth. J. Sea Res.* **12,** 164–179.

Reijnders, P. J. H. (1979). Organochlorine and heavy metal residues in harbour seals of Schleswig-Holstein plus Denmark and the Netherlands: Their possible effects in relation to reproduction in both populations. *ICES CM 1979/N*: 18.

Reijnders, P. J. H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **324,** 456–457.

Roberts, J. R., deFreitas, A. S. W. & Gidney, M. A. I. (1977). Influence of lipid pool size on bioaccumulation of the insecticide chlordane by Northern Redhorse Suckers (*Moxostoma macrolepidotum*). *J. Fish. Res. Bd. Can.* **34,** 89.

Robinson, R. & Roberts, M. (1968). Accumulation, distribution and elimination of organochlorine insecticides by vertebrates. *S. C. I. Monogr.* **No. 29,** 106.

Robinson, R., Baldwin, M. & Walker, A. I. T. (1969). The pharmokinetics of HEOD (Dieldrin) in the rat. *Food Cosmet. Toxicol.* **7,** 317.

Safe, S. (1984). Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *CRC Critical Reviews in Toxicology* **13,** 319–395.

Safe, S., Bandiera, S., Sawyer, T., Robertson, L., Safe, L., Parkinson, A., Thomas, P. E., Ryan, D. E., Reik, L. A., Levin, W., Denomme, M. A. & Fujita, T. (1985). PCBs: Structure-function relationships and mechanisms of action. *Env. Health Persp.* **60,** 47–56.

Schneider, R. (1980). Significance of equilibrium partitioning and lipid composition in PCB accumulation by fish. *ICES CM 1980/E*: 12.

Schneider, R. (1982). Polychlorinated biphenyls (PCBs) in cod tissues from the western Baltics: Significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. *Meeresf.* **29,** 69–79.

Schulz, D. E., Petrick, G. & Duinker, J. C., (1989). Complete characterisation of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environ. Sci. Technol.* In press.

Sipes, I. G., Slocumb, M. L., Perry, D. F. & Carter, D. E. (1980). 4,4′-dichlorobiphenyl: distribution, metabolism and excretion in the dog and the monkey. *Toxicol. Appl. Pharmacol.* **55,** 554–563.

Sokal, R. R. & Rohlf, F. J. (1969). Biometry. The principles and practice of statistics in biological research. W. H. Freeman & Company, San Francisco, 776 pp.

Tanabe, S., Tatsukawa, R., Tanaka, H., Maruyama, K., Miyazaki, N. & Fujiyama, T. (1981a). Distribution of total burdens of chlorinated hydrocarbons in bodies of striped dolphins (*Stenella coeruleoalba*). *Agric. Biol. Chem.* **45,** 2569–2578.

Tanabe, S., Tanaka, H., Maruyama, K. & Tatsukawa, R. (1981b). Ecology and bioaccumulation of Stenella coeruleoalba: elimination of chlorinated hydrocarbons from mother striped dolphins through parturition and lactation. In: *Studies on the levels of organochlorine compounds and heavy metals in marine organisms.* (ed. T. Fujiyama). Univ. of Ryukyus, Okinawa.

Tanabe, S., Watanabe, S., Kan, H. & Tatsukawa, R. (1988). Capacity and mode of PCB metabolism in small Cetaceans. *Mar. Mamm. Sci.* **4,** 103–124.

Tanabe, S., Tatsukawa, R., Maruyama, K. & Mitazaka, N. (1982). Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. *Agric. Biol. Chem.* **46,** 1249–1254.

Verwey, J. (1975). The Cetaceans *Ph. phoceoena* and *Tursiops truncatus* in the Marsdiep area (Dutch Wadden Sea) in the years 1931–1973. *Neth. Ins. Sea Res. Repts.* No. 1975-17a-17b.

Wagemann, R. & Muir, D. C. G. (1981). Assessment of heavy metals and organochlorine concentrations in marine mammals of northern waters. *ICES CM 1981/N*: 9.

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Normal and aberrant origin and course of the coronary arteries in small cetaceans

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Summary

The normal and aberrant origin and course of the coronary arteries in 27 small cetaceans is described. All hearts display a right preponderance of the coronary arteries. Five anomalies of the arteria coronaria sinistra and one of the ramus circumflexus a.c.s. were observed. None of them caused the death of the animal. The developing of full functional arteries from the anastomoses between the coronary arteries may be the saving factor.

Introduction

Anomalous origin and aberrant courses of the coronary arteries have been observed in man (El-Said *et al.*, 1973) as well as in domestic animals (v.Nie, 1968). The incidence in these mammals remains unknown. In cetaceans only one anomaly has previously been recorded, in a *Physeter* (Truex *et al.*, 1961), while in *Phoca vitulina,* 19 cases have been observed (v.Nie, 1985). Here too an exact incidence mark cannot be computed.

Since a study of the anomalous localization or absent origin and aberrant courses of the coronary arteries cannot be performed without a study of the normal topography of these structures. Both will be described in the following communication.

The literature concerning the cetaceans available to the present author is reviewed in Table 1.

The nomenclature of the morphological structures used will be derived from the Nomina Anatomica Veterinaria, third edition, 1983.

Material and techniques

The hearts belonged to 12 dolphins: 6 white-snouted dolphins (*Lagenorhynchus albirostris,* Gray, 1846); 1 white-side dolphin (*Lagenorhynchus acutus,* Gray, 1828); 3 true dolphins (*Delphinus delphis,* L., 1758); 2 bottle nosed dolphins (*Tursiops truncatus,* Montagu, 1821) and 15 harbour porpoises (*Phocoena phocoena,* L., 1758).

The hearts were rinsed in tapwater after the post-mortem examination of the animals and stored in 4% formaldehyde for at least one month. Since the clotted blood in the coronary arteries makes an X-ray examination not practicable, the dissection of the vessels was performed manually by means of fine polished surgical instruments under a spotlight.

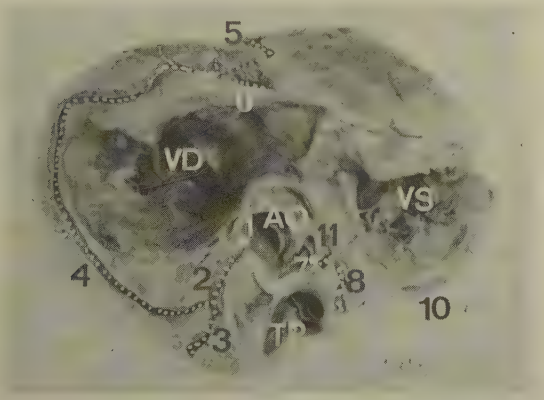

Figure 1. Basal view of the heart of a dolphin with an aplasia of the origin of the r.interv.paraconalis.

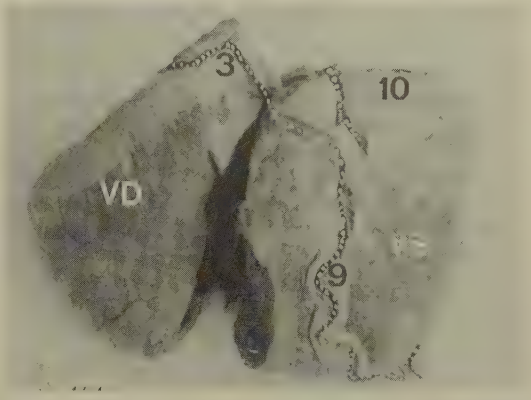

Figure 2. View of the left side of the heart of a dolphin with an aplasia of the origin of the r.interv.paraconalis.

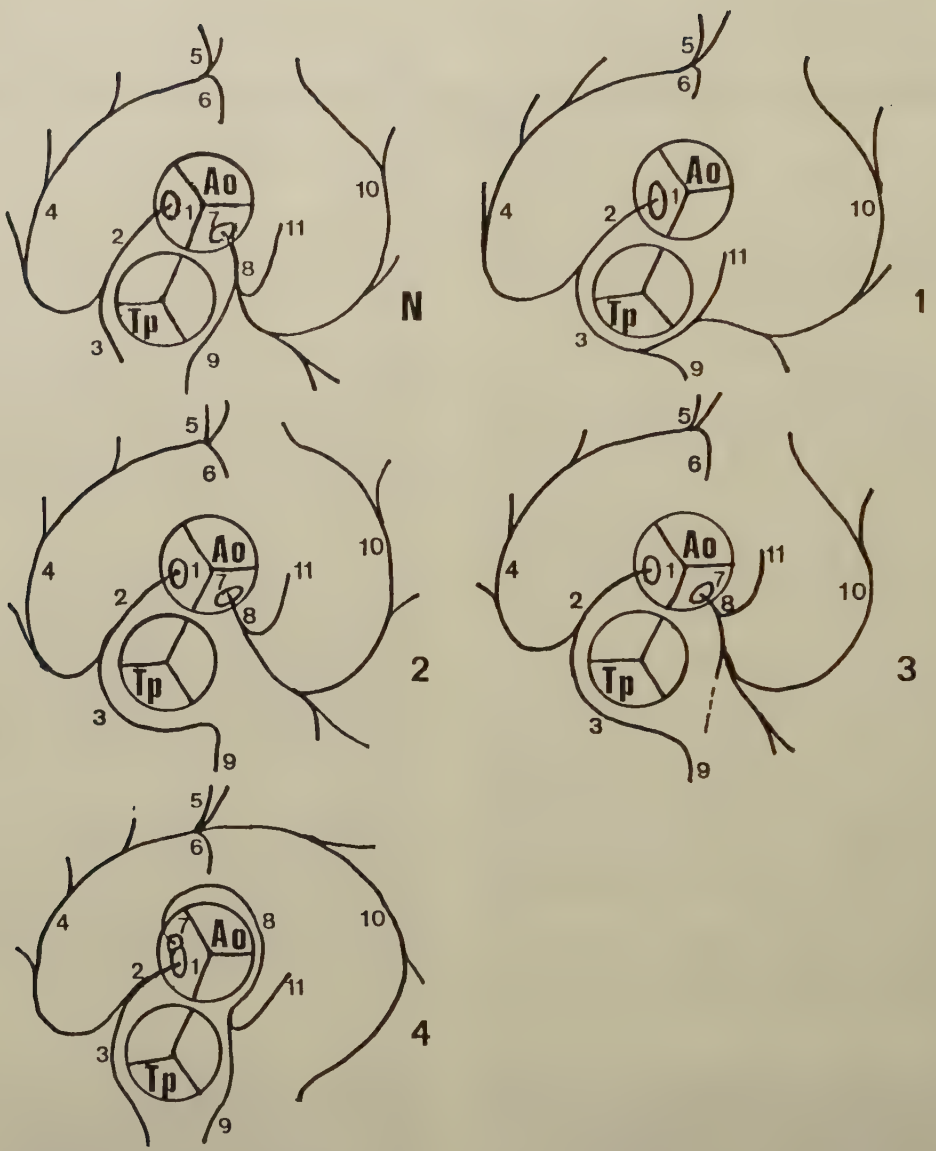

Figure 3. N. normal origin and course of the arteriae coronariae. 1. aplasia origin of the a.c.s. 2. aplasia origin of the ramus interventricularis paraconalis. 3. hypoplasia origin of the ramus interventricularis paraconalis. 4. aberrant origin a.c.s. and aplasia of the ramus circumflexus a.c.s.

Ao—Aorta
VD—ventriculus dexter
TP—truncus pulmonalis
VS—ventriculus sinister
1. origin arteria coronaria dextra
2. arteria coronaria dextra (a.c.d.)
3. ramus conalis
4. ramus circumflexus a.c.d.
5. ramus interventricularis subsinuosus
6. ramus septalis
7. origin arteria coronaria sinstra
8. arteria coronaria sinistra (a.c.s)
9. ramus interventricularis paraconalis
10. ramus circumflexus a.c.s.
11. ramus atrialis

====course of the arteria coronaria

n.b. according to Nomina Anatomica Veterinaria 1983

Table 1.

Year	Author	Species	R/L prep.	Anast.	Anomalies
1901	Marschner	Phocoena 4x	R/L	+	
		Balaenoptera 3x	R/L	+	
1938	Walmsley	Balaenoptera 1x	R	+	
1959	Race *et al.*	Physeter 2x	R	+	
1961	Truex *et al.*	Balaenoptera 3x	R	+	
		Eschrichtius 2x	L	+	
		Physeter 5x	?	+	aplasia orig. a.c.s. 1x
1968	Sommer *et al.*	Tursiops 4x	R	+	
1975	Rowlatt *et al.*	Phocoena 36x	R	–	
1977	Cave	Tursiops 1x	R	–	
1978	Halina *et al.*	Phocoena 16x	R	+	
1988	this paper	Phocoena 15x	R	+	aplasia orig. a.c.s. 2x err. orig. a.c.s. 1x
		Lagenorhinchus 7x	R	+	aplasia orig. a.c.s. 1x aplasia orig. r. interv. parac. 1x
		Delphinus 3x	R	+	hypoplasia r. interv. parac. 1x
		Tursiops 2x	R	+	

For legends, symbols and abbreviations see table 2

Results

The results are listed in Table 2 and are displayed in the diagrams and the photographs.

The results may be summarized as follows: The ramus interventricularis subsinuosus is drained by the arteria coronaria dextra (abridged a.c.d.), so a right preponderance of the coronary arteries is acceptable. The following aberrant courses and origins of the arteria coronaria sinistra (abridged a.c.s.) were observed:

a. Thrice, an aplasia of the origin of the a.c.s. (once in a dolphin and twice in harbour porpoises).
b. Once an aplasia of the origin of the ramus interventricularis paraconalis (in a dolphin).
c. Once a hypoplasia of the ramus interventricularis paraconalis (in a dolphin).
d. Once an aberrant origin of the a.c.s. and aplasia of the left ramus circumflexus a.c.s. (in a harbour porpoises).

Discussion

The described cases of aberrant and anomalous origin and courses of the coronary arteries in aquatic mammals were obviously compatible with life. The observed anastomoses secured by the development in full functional arteries a correct circulation. The right preponderance of the a.c.d. plays an important role too, while the greatest and most important part of the mentioned circulation is provided by this artery.

Up till now an aplasia of the origin and a hypoplasia of the a.c.d. and its branches has not been observed in aquatic mammals. Such cases are incompatible with life hypothetically; they may be observed in dead baby animals.

The observed cases display the same variants as described in seals (v.Nie, 1985); however in these animals a left preponderance of the a.c.s. is present in 5% of the cases. The scant anastomses in the seal heart make this heart more vulnerable than that of the cetaceans, in cases where anomalous or aberrant coronary arteries are found.

The pathology of the mentioned anomalies is still in discussion in terrestrial animals (v.Nie, 1968), while in man anomalous coronary arteries are taken up in surgical programmes (El-Said *et al.*, 1973).

Recently Moscovici (1985) states, that the right preponderance as well as the left one of the heart circulation is based upon macroscopical observations only. His special technique for visualizing the minor intra-muscular coronary arteries leads to the conclusion, that in man a left preponderance is always present. It is the present author's opinion, that Moscovici's technique will be valuable in a further study of the topography of the coronary arteries in small cetaceans.

Conclusion

A right preponderance of the coronary arteries was observed in 27 examined cetacean hearts.

Table 2.

Nr	Species	Heart weight	R/L prep	Anast.	Anomaly
1	P. pho	60	R	+	
2	T. tru	80	R	+	
3	P. pho	85	R	+	
4	T. tru	90	R	+	
5	P. pho	100	R	+	aberrant orig. a.c.s.
6	P. pho	125	R	+	hypopl. r. circum. a.c.s. 4
7	P. pho	150	R	+	
8	P. pho	150	R	+	
9	P. pho	170	R	+	
10	P. pho	180	R	+	
11	P. pho	235	R	+	
12	P. pho	250	R	+	aplas. orig. a.c.s. 1
13	P. pho	250	R	+	aplas. orig. a.c.s. 1
14	P. pho	285	R	+	
15	P. pho	320	R	+	
16	P. pho	320	R	+	
17	P. pho	380	R	+	
18	D. del	550	R	+	
19	D. del	550	R	+	hypopl. r. interv. par. 3
20	L. alb	675	R	+	
21	D. del	750	R	+	
22.	L. acu	1300	R	+	
23.	L. alb	1350	R	+	
24	L. alb	1800	R	+	
25	L. alb	2525	R	+	aplas. orig. r. inter. par. 2
26	L. alb	2800	R	+	aplas. orig. a.c.s. 1
27	L. alb	2800	R	+	

D. del: Delphinus delphis L. 1758
L. acu: Lagenorhinchus acutus Gray 1825
L. alb: Lagenorhynchus albirostris Gray 1828
P. pho: Phocoena phocoena L. 1758
T. tru: Tursiops truncatus Montagu 1821
R/L prep: R or L preponderance, ramus interventricularis subsinuosis is drained by a.c.d. (R) or a.c.s. (L)
1, 2, 3 and 4 refer to the diagrams 1, 2, 3 and 4
anast.: anastomoses; a.c.d.: arteria coronaria dextra; a.c.s.: arteria coronaria sinistra; aplas.: aplasia; hypopl.: hypoplasia; orig.: origin; r.interv.par.: ramus interventricularis paraconalis

Anomalous course of these arteries—6 cases—was restricted to the origin and course of the a. coronaria sinistra and her main branches.

None of these anomalies caused the death of the animal.

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References

Cave, A. J. E. (1977). The Coronary Vasculature of the Bottlenosed Dolphin (Tursiops truncatus). In: Functional Anatomy of Marine Mammals, vol. 3, ed. R. J. Harrison, Acad. Press, London, New York & San Francisco, 119–215.

El-Said, G. M., Dawson, J. T. jr., Sandiford, F. M., Mullins, C. E., Hallman, G. L., Cooly, D. A. & McNamara, D. G. (1973). Coronary artery anomalies. *Eur. J. Cardiol.* **1,** 63–70.

Halina, W. G. & Gaskin, D. E. (1978). Functional anatomy of the coronary system of the harbour porpoise, Phocoena phocoena, (L). *Can. J. Zool.* **56,** 1643–1653.

Marschner, L. (1901). Beiträge zur Anatomie und Physiologie des Herzens und der grossen Gefässstämme der Wasser-säugetiere. Inaugural Dissertation, Breslau.

Nie, C. J. van (1968). Anomalous Origin of the Coronary Arteries in Animals. *Path. Vet.* **6,** 313–326.

Nie, C. J. van (1985). The topography of the coronary arteries in the Common Seal (Phoca vitulina vitulina) *Aquatic Mammals* **11,** 66–68.

Race, G. J., Edwards, W. L. J., Halden, E. R., Wilson, H. E. & Luibel, F. J. (1959). A Large Whale Heart. *Circulation XIX,* 928–932.

Rowlatt, U. & Gaskin. D. E. (1975). Functional Anatomy of the Heart of the Harbour Porpoise, Phocoena phocoena. *J. Morphol.* **146,** 479–494.

Sommer, L. S., McFarland, W. L., Gallino, R. E., Nagel, E. L. & Morgane, P. J. (1968). Hemodynamic and coronary angiographic studies in the bottlenose dolphin (Tursiops truncatus). *Am. J. Physiol.* **215,** 1498–1505.

Truex, R. C., Nolan, F. G., Truex, R. C. jr., Schneider, H. P. & Perlmutter, H. I. (1961). Anatomy and Pathology of the Whale heart with Special Reference to the Coronary Circulation. *The Anatomical Rec.* **141,** 325–354.

Walmsley, R. (1938). Some observations on the vascular system of the fetal female finback. *Contrib. Embryol.* **27,** 107–178.

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The olfactory regions in the nose of the harbour porpoise *Phocoena phocoena* (Linne, 1758)

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Zusammenfassung

Die Riechzonen in der Nase des Schweinswals *Phocoena phocoena* (Linne, 1758).

Weil der Riechnerv (*Bulbus olfactorius*) bei den Zahnwalen vollkommen zurückgebildet ist, ging man bisher davon aus, daß auch der Geruchssinn verloren gegangen ist. Nun wurden in den Nasen von Schweins-walen Riechzonen (*Regio olfactoria*) mit funktions-fähigen Riechzellen gefunden, deren Sinnesgeißeln als Chemorezeptoren identifiziert werden konnten. Daraus kann postuliert werden, daß die Schweinswale doch in der Lage sind, bestimmte chemische Verbindungen mit der Nase zu erfassen.

Introduction

'The anatomy of the odontocete brains indicates that they can taste but cannot smell. Apparently, when the blowhole migrated to the top of the head, the neural pathways for smell were sacrificed' (Caldwell & Caldwell, 1972). Today it is generally accepted that toothed whales are unable to smell because of the above mentioned observation.

However in the nose of three harbour porpoises a large number of chemoreceptor cells have been discovered during a histological investigation of the olfactory regions. Why are the olfactory regions and olfactory cells retained, if at the same time the olfactory nerves are sacrificed? What is the function of such cells?

Materials and methods

To study the olfactory regions, three harbour porpoises heads were examined. The three animals were caught as bycatches in the North and Baltic Seas. Short time after death they were deep frozen. In this condition the heads were sectioned into slices of some millimetres in thickness.

These sections were fixed with formalin under vacuum at a lower temperature. After this, the sections of two heads were embedded in paraffin, and the sections of one head was prepared using the plastination method by resins (von Hagens, 1979). Selected parts of the paraffin embedded material were cut into histological sections.

Findings

After death mucous layers disintegrate very quickly, and along with the mucosa the olfactory cells get lost. Therefore, in all harbour porpoise noses examined, the olfactory regions were only partly preserved. They were found in the vestibular sac (*Saccus vestibularis*) and in the frontal sac (*Saccus frontalis*) (Fig. 1). Olfactory cuticles are situated on the folded integument on the floor of the vestibular sac (Fig. 2), as well as in the frontal sac where they cover the

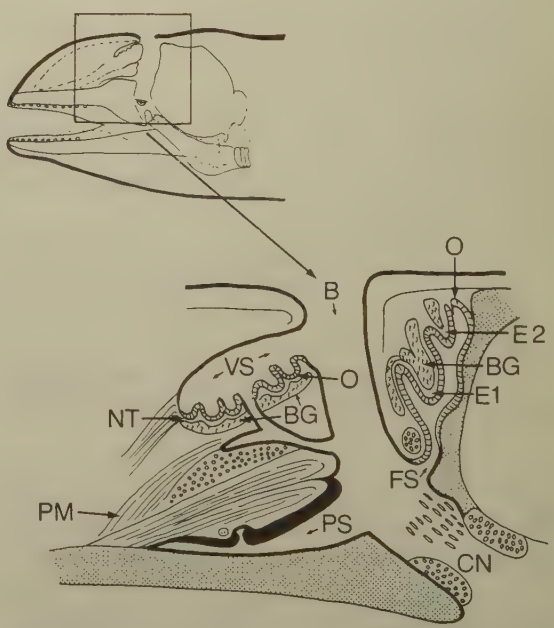

Figure 1. Schematic section through the nose of the harbour porpoise. Blowhole (B), Bowman-glands (BG), ethmoidal (E), frontal sac (FS), nosehole (CN), nasoturbinal (NT), plugmuscle (PM), premaxillary sac (PS), vestibular sac (VS), olfactory cuticles (O).

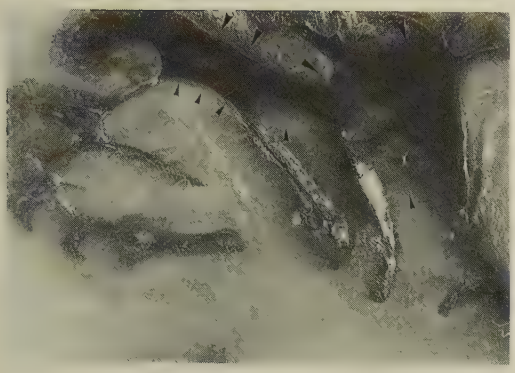

Figure 2. Longitudinal section through the vestibular sac, magnification 10×. The different cuticles of the sac are clearly distinguishable. The integument above is dark like the epidermis. At the bottom of the sac the integument is thin and has a striped olfactory cuticle.

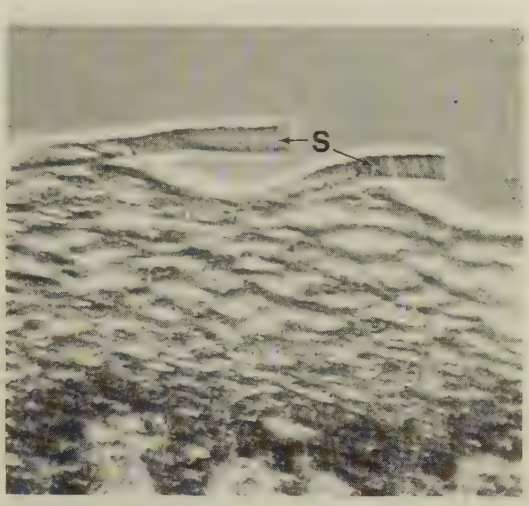

Figure 4. View of the outside of the retina, magnification 100×. The mucosa is lost and only the relics of two olfactory cells (S) are preserved.

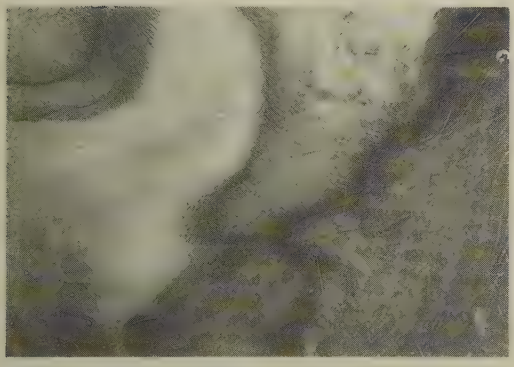

Figure 3. Section through a fold in the bottom of the vestibular sac with a view of the cells of the olfactory region, magnification 25×.

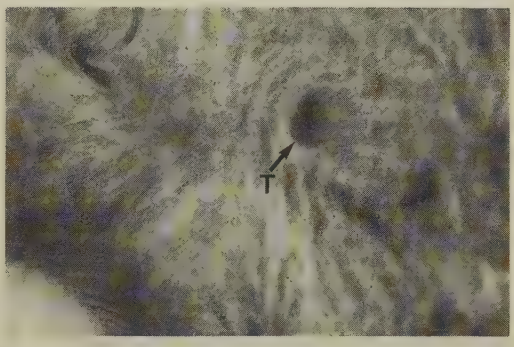

Figure 5. Section through the Bowman-gland below the frontal sac, magnification 25×. The opening (T) leads to the splash nozzles.

ethmoturbinals 1 and 2 (Fig. 6). The third ethmoturbinal is sacrificed. The olfactory regions differ from the respiratory regions by their colour and morphology (Fig. 2). The olfactory regions are yellowish brown and the mucosa is thicker than the dark mucosa of the respiratory cuticles. The brownish colour originates from pigment corpuscles (Melanosome) which are situated in the base of the olfactory cuticle. The yellowish colour is caused by cell fluids. The base of the olfactory cuticle is arranged in a network similar to the retina in the eye (Fig. 3, Fig. 4). Splash nozzles protrude from the cuticle; they are the openings of the Bowman-glands which extend below the whole olfactory regions (Fig. 5). The retina-like cuticle is covered with a layer of olfactory cells which are protected by the thick mucosa (Fig. 6).

The olfactory cells have a longitudinal shape with a length of up to 100 μ and a diameter of nearly 12 μ. The nucleus of a cell is very large and surrounded by Golgi-complexes. Neurites pass through the cell and end up in the sense whip. The sense whips have a length of nearly 25 μ. Their walls have many pores like chemoreceptors of other vertebrates which are adapted to living in water (Fig. 7, Fig 8).

Discussion

The evolutionary migration of the blowhole included a transfer of the olfactory regions (Gruhl, 1911). Nowadays they are located in the integuments of the

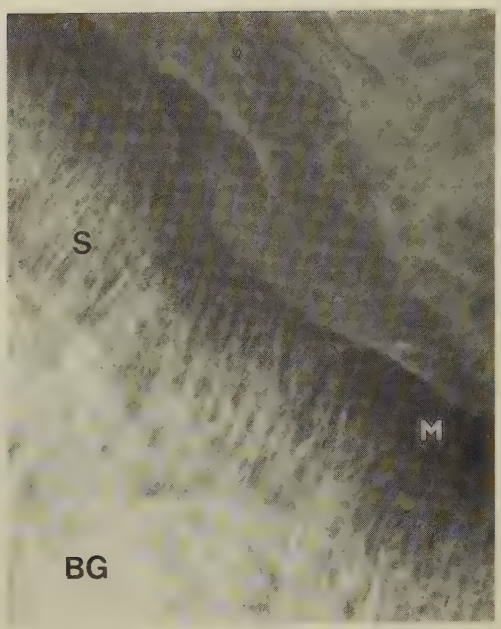

Figure 6. Section through the olfactory region in the frontal sac, magnification 50 × . The region is covered with a thick mucous layer. The olfactory cells (S) are clearly recognizable in the well preserved mucosa (M). Below the olfactory cuticle is situated the Bowman-gland (BG).

frontal sac and the vestibular sac. The olfactory region in the frontal sac is comparable with the ethmoturbinals, and the olfactory region in the vestibular sac is comparable with the naso-turbinal (*Turbinale nasalis*) of other mammals. The olfactory epithelia, the stock of distinctive chemoreceptory cells and the position of the Bowman-glands below the olfactory regions' support Gruhl's (1911) hypothesis that toothed whales could have an olfactory sense.

Experiments with toothed whales to check their reactions to chemical compounds are described, but we still know very little about dolphin chemoreception (Nachtigall, 1986).

As mucous epithelia decays very quickly after death, olfactory cells are destroyed along with the mucosa, and relics of the cuticles were presumably overlooked. In assuming that the bulbi of the olfactory nerves (*Bulbus olfactorius*) were sacrificed, Kückenthal (1893), Radwitz (1900), Boenninghaus (1911) and Caldwell and Caldwell (1972) stated that toothed whales are unable to smell.

Because the harbour porpoises were quickly deep frozen after their decease, large parts of the olfactory regions were preserved. By the careful fixation at low temperatures under vacuum, the cells appear well preserved. The well preserved cellular organelles and the form of the cells demonstrate that they had a real function.

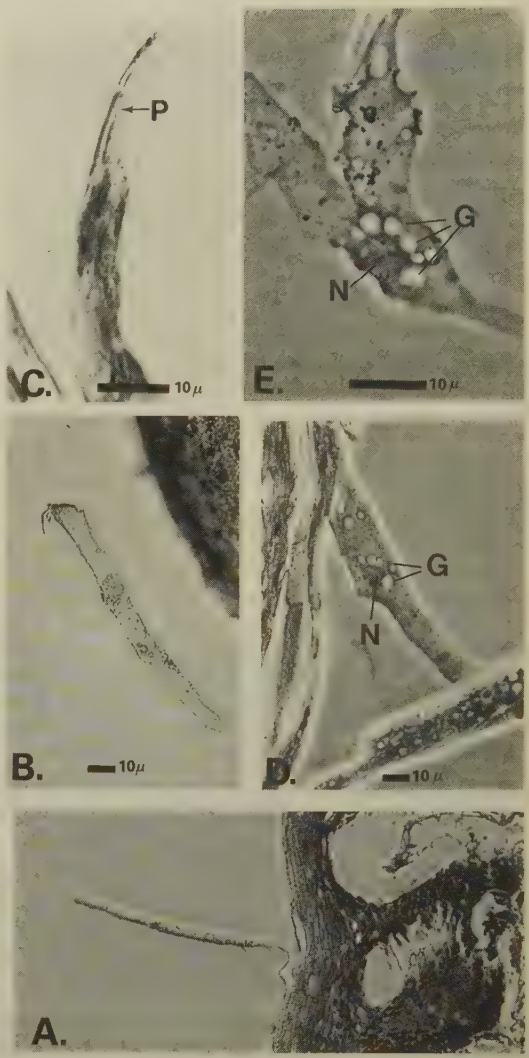

Figure 7. Details of the olfactory cells. A. The olfactory cell originates between the cell layer above the retina. B. A separate olfactory cell with a sense whip. C. Sense whip with pores (P), which are characteristic for chemoreceptors. D. The middle part of the olfactory cell with the cell nucleus (N), surrounded by vesicles (G). E. The nucleus (N) with Vesicles (G).

All olfactory cells which were found were innervated. The existence of sense-hairs with pores, which is a characteristic for chemoreceptors, proved this. It follows that harbour porpoises are able to detect chemical particles.

However, the preservation of olfactory regions during evolution seems paradoxical in case the olfactory nerves have disappeared. On the other hand, the well preserved innervated olfactory cells do indicate that they have a function. Human beings whose

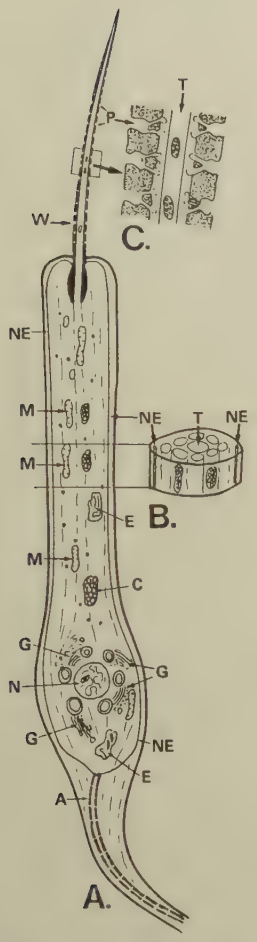

Figure 8. Model of one olfactory cell *A.* with a transverse section through the cell *B.* and a longitudinal section through the whip *C.* Axon (A), cyclocyst (C), endoplasmatical reticulum (E) Golgi complex with vesicles (G), mitochondria (M), nucleus (N), neurite (NE), pore (P), tubulus (T), sense whip (W).

olfactory nerves have been destroyed are able to take up odours from chemical compounds of nitrate, ammonia and sal ammoniac (Schiefer *et al.*, 1986).

Those odours are taken up by the nerve-ends of the trigeminal nerve. The branches of the trigeminus (*Rami nasales interni*; *Rami terminalis*) lead to the olfactory region of the brain (Rauber & Kopsch, 1953; Corning, 1942). It may therefore be possible that the harbour porpoise nose has a function similar to a destroyed nose of a human being. This would explain the presence of the well preserved olfactory cells.

References

Boenninghaus, G. (1903). Der Rachen von Phocoena communis Less. Die Funktion der Nasensäcke oder der Nasen-höhlen. Zoologische Jahrbücher, **B17:** 1–95. Verl. G. Fischer Jena.

Caldwell, D. K. and M. C. Caldwell (1972). Sense and communication. In: Mammals of the sea, 466–502, Ed. by S. H. Ridgway. C. C. Thoma publisher, Springfield, Illinois, U.S.A.

Corning, H. K. (1942). Lehrbuch der topografischen Anatomie, 402. Verl. J. F. Bergmann, München.

Gruhl, K. (1911). Beiträge zur Anatomie und Physiologie der Cetaceennase. Jenaer Zeitschrift für Naturwissenschaften, **47(3),** 367–414.

Hagens, G. von (1979). Emulsifying resins for plastination. Der Präparator, **25(2):** 43 50, Bochum.

Hirsch, G. C., H. Ruska und P. Sitte (1973). Grundlagen der Cytologie, 790. Gustav Fischer Verlag, Stuttgart.

Kückenthal, W. (1893). Vergleichende-anatomische und entwicklungs-geschichtliche Untersuchungen an 'Wahltieren: Bau und Entwicklung der Cetaceennase. Denkschrift der Medizinischen und Naturwissenschaftlichen Gesellschaft Jena, **3(2):** 322–349.

Nachtigall, P. E. (1986). Vision, audition and chemoreception in dolphins and other marine mammals. In: Dolphin cognition and behaviour 79-113, Ed. by R. Schustermann, J. A. Thomas and F. G. Wood. L. Erlbaum Associates, Inc. Publisher, Hillsdale, New Jersey.

Radwitz, B. (1900). Die Anatomie des Kehlkopfes und der Nase von Phocoena communis Cuv. Internationale Monatszeitschrift für Anatomie und Physiologie, Vol. **17:** 245–254.

Rauber, R. K. und Fr. Kopsch, 1953: Lehrbuch und Atlas der Anatomie des Menschen B. III, 312. Verl. G. Thieme, Leipzig.

Schifer, P., S. Peiper und R. Schneider (1986). Lehrbuch der Histologie, 681. Springer Verlag, Berlin. Heidelberg, New-York, London.

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